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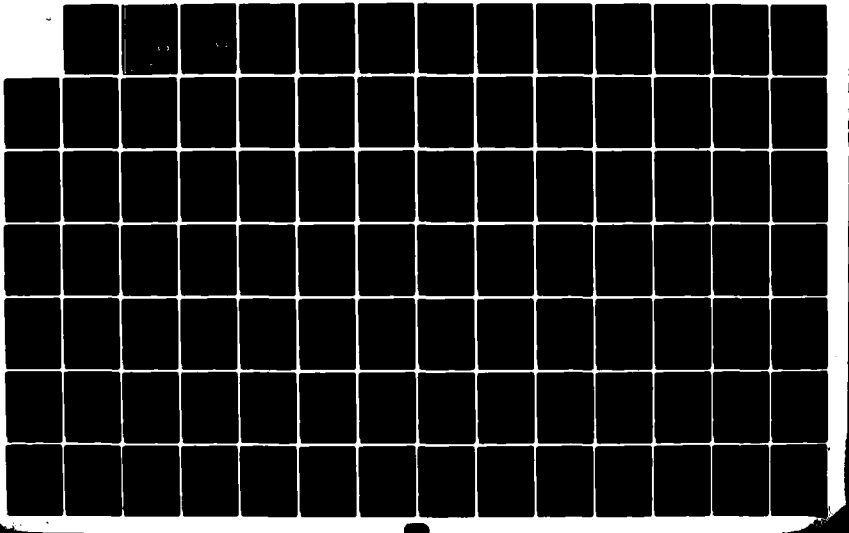

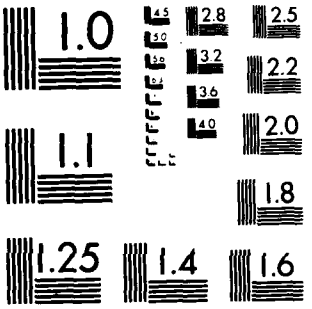

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A STUDY ON THE GASTROINTESTINAL HORMONES AND THE GASTRIC ACID SECRETION DURING PHYSICAL STRESS IN MAN

BY

OLAV ØKTEDALEN

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In the present thesis, we have studied the effect of prolonged physical stress and absolute fasting on the blood levels of gastrointestinal peptides and the secretion of gastric acid. Especial attention has been paid to peptide levels that influence, or are influenced by, the secretion of gastric acid (gastrin, secretin, pepsinogens I), or that probably have metabolic function (vasoactive intestinal polypeptide, pancreatic polypeptide). The hyperchlorhydria was not caused by the gastrin hormone, but the hypersecretion of gastric acid could account for approximately fifty per cent of the hypersecretinemia found during the stress. High plasma levels of secretin were also found during a 4-5 day period of absolute fasting. The high plasma levels of vasoactive intestinal polypeptide (VIP) were not influenced by the gastric acid secretion. Instead, the results indicated that VIP is "a peptide of substrate need". The high fasting serum levels of human pancreatic polypeptide were modified by nutrient ingestion both during physical stress and absolute fasting.

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PREFACE

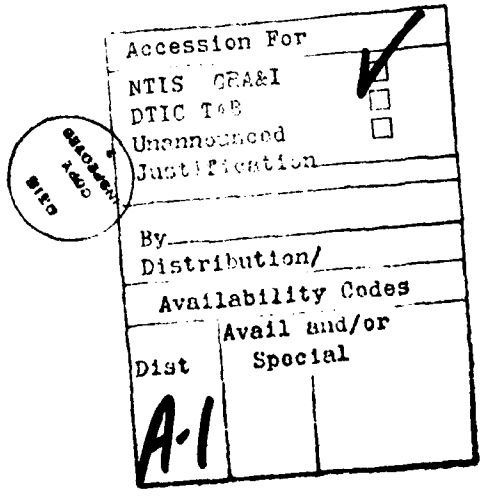
The present work was carried out at the Norwegian Defence Research Establishment, Division for Toxicology, Kjeller, in the years 1979-1982. It represents a part of a continuing research program with the purpose to elucidate the effects of prolonged physical stress on physiological parameters in man. In the hectic life of a modern society we are subjected to several forms of stress. Knowledge of how the organism reacts during such circumstances is a prerequisite for understanding the complaints of stress-mediated symptoms, how to treat them, and how to prevent the development of illness. Ulcer disease has in many ways been considered a stress-induced psychosomatic illness. Therefore, it was of interest to investigate what influence sustained physical stressors exert on the gastrointestinal tract with especial attention to the gastrointestinal hormones and the gastric acid secretion.

I am in deep gratitude to Dr F Fonnum, the head of the Division of Toxicology, for encouraging and introducing me to scientific work in the project. I am grateful to major general T Kluge for his trust and support in my study. I am indepted to my collaborator P K Opstad for valuable help and inspiration during the work, and to J Fahrenkrug and O Flaten who advised me in radioimmunoassay methodic. I appreciate the cooperation with the Norwegian Military Academy, its leader, colonel A Pran, and the officers and cadets participating in the courses. I want to thank Ms E Eliassen and Ms B Andersen who gave me excellent technical assistance during most of the study. Thanks is also offered to the secretaries T Thorsen and K Skovli who typed most of the manuscripts and to M Ekstrand and P J Karlsen for drawing the figures. Special thanks is diverted to my wife for patience during the work. The study was supported by the Norwegian Joint Medical Service who provided a research fellowship.

Kjeller, December 1983

Olav Øktedalen

To my surprise



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A STUDY ON THE GASTROINTESTINAL HORMONES AND THE GASTRIC ACID SECRETION DURING PHYSICAL STRESS IN MAN

SUMMARY

In the present thesis we have studied the effect of prolonged physical stress and absolute fasting on the blood levels of gastrointestinal peptides and the secretion of gastric acid. Especial attention has been paid to peptide levels that influence, or are influenced by, the secretion of gastric acid (gastrin, secretin, pepsinogens I), or that probably have metabolic function (vasoactive intestinal polypeptide, pancreatic polypeptide). The hyperchlorhydria was not caused by the gastrin hormone, but the hypersecretion of gastric acid could account for approximately fifty per cent of the hypersecretinemia found during the stress. High plasma levels of secretin were also found during a 4-5 day period of absolute fasting. The high plasma levels of vasoactive intestinal polypeptide (VIP) were not influenced by the gastric acid secretion. Instead, the results indicated that VIP is "a peptide of substrate need". The high fasting serum levels of human pancreatic polypeptide were modified by nutrient ingestion both during physical stress and absolute fasting.

1 GENERAL INTRODUCTION

1.1 Definition of stress

More than thirty years have elapsed since the well known expert on stress, Dr Hans Selye, introduced his "adaptation syndrome" (1), and considered stress as the reaction of the organism to maintain internal homeostatic balance when exposed to changes. According to his definition, the stress reaction is specific in its manifestation independant of the quality of the causing factors, the stressors. Stress has obviously its good points as well as bad ones, and a life without stress would be a colourless existence. How we react to stressors is highly individually and linked to our personality and previous experiences. The key issue in the definition of stress by Selye is how we adapt to the new situation. The difference in perception of stressors between different individuals has really made it difficult

to evaluate the effects of causing stress factors. The lack of quantitative methods used to assess stressors has for a long time prevented any analysis of their importance in epidemiological studies.

1.2 Stress and gastric disease

Some situations are undoubtedly so threatening and so unpleasant that a few persons would adapt to them completely. Results of investigations from such stressful events have shown that stressors can break down mental and physical health. Experiences from war times show that soldiers in combat often react with anxiety, depression, lack of sleep, nightmares and anorexia. Furthermore, wartime experience shows an increased prevalence of hypertension in soldiers exposed to life-threatening situations (2), and it is presumed that environmental stress factors may contribute to the development of the illness of essential hypertension (3), a major health problem in the modern society. In addition, there are reports on increased incidence of peptic ulcer in man during wartime (4), and gastric stress ulceration has been a major cause of morbidity and mortality in soldiers during combat (5).

Peptic ulcer has been regarded a psychosomatic disease, a disease caused by the modern society. Cooper (6) has reported that psychosomatic reasons account for about 18 per cent of the peptic ulcers, while a study in identical twins (7) suggests that environmental factors and hereditary factors each contributes approximately 50 per cent to the development of ulcer disease. However, the number of stressful life events in healthy man (8), and in chronic gastric ulcer patients (9, 10) as well as in chronic duodenal ulcer patients prior to the first symptoms of ulcer (11), are similar. Furthermore, there is no difference in the quantified scores for changes or suffering caused by the events (10, 11). These negative findings indicate the importance of obtaining a description of the personality of the ulcer patient, his defence mechanisms and other personal characteristics. Reports claim that the duodenal ulcer patient is more tensed (12), anxious (13), neurotic (13), depressed (14) and selfsufficient (15) than healthy man. Perhaps this could explain why the peptic ulcer patient

is said to be more sensitive to stressors (16, 17), and that he experiences stressful life events more frequently than non-ulcer subjects (15, 18). Most investigators seem to agree that peptic ulcer disease is a heterogenous group of disorders where the psychosocial factors must be accounted for in their pathogenesis.

1.3 The object of the investigation

The present thesis was undertaken to investigate what influence physical stressors exert on the secretion of gastric acid and on the blood levels of gastrointestinal peptides that probably have control function in the digestive process. Since peptic ulcer disease in many ways has been linked to an increase in the gastric acid, we decided to measure peptide levels that are known to regulate the gastric acid secretion (gastrin), and that are influenced by the amount of gastric acid produced (secretin, group I pepsinogens). Our subjects were in caloric deficit, and we also found it of interest to measure the blood levels of gastrointestinal peptides that have possible metabolic influence (vasoactive intestinal polypeptide and human pancreatic polypeptide).

Two experimental models were used. In one model, well-trained, healthy men (between 20 and 30 years of age) were exposed to a 4-5 day period of heavy physical exercise (approximately 35 per cent of their maximal oxygen uptake), combined to calorie supply deficiency (intake of approximately 6300 kJ/24 h against a combustion of about 36000-43000 kJ/24 h) and severe sleep deprivation (about 2-4 h of sleep during four to five days as a total).

In the other model, healthy subjects (ranging from 20 to 40 years of age) were exposed to a 4-5 day period of absolute fasting while performing their daily work at a research laboratory.

More specifically the present work was carried out to:

a) evaluate the effect of combined prolonged physical exercise, severe calorie deficiency, and excessive sleep deprivation on the basal and the nutrient-stimulated blood levels of gastrin, human

pancreatic polypeptide (hPP), secretin, vasoactive intestinal polypeptide (VIP), and group I pepsinogens (PGI) (I, II, III, V)

1) the effect of additional calories (II, III, V)
2) the effect of additional sleep and rest (II, III, V)

b) evaluate the influence of pure calorie deficiency on the fasting and the nutrient-stimulated blood levels of these peptides (VI, VII)

c) investigate how these physical stressors influence the unstimulated and the stimulated gastric acid secretion (IV, VIII)

d) investigate how the blood levels of gastrin, secretin and VIP are influenced by the gastric acid produced during such a stress period (IV)

e) measure the effect of physical stress on the pure vagal stimulation of gastric secretion and gastrointestinal peptide levels (VIII)

The results are presented in eight separate publications:

Paper I — hPP and gastrin response to a liquid meal and oral glucose during prolonged severe exercise, caloric deficit, and sleep deprivation.
O Oektedalen, O Flaten, P K Opstad & J Myren, Scand J Gastroenterol 17, pp 619-624, 1982.

Paper II — Secretin - a new stress hormone?
O Oektedalen, P K Opstad and O B Schaffalitzky de Muckadell, Regulatory Peptides 4, pp 213-219, 1982.

Paper III — Plasma concentration of vasoactive intestinal polypeptide during physical exercise, calorie supply deficiency, and sleep deprivation.
O Øktedalen, P K Opstad, J Fahrenkrug and F Fonnum, Scand J Gastroenterol 18, pp 1057-1062, 1983.

Paper IV — Basal hyperchlorhydria and its relation to the plasma concentrations of secretin, vasoactive intestinal polypeptide (VIP) and gastrin during prolonged strain.
O Oektedalen, P K Opstad, O B Schaffalitzky de Muckadell, O Fausa and O Flaten, Regulatory Peptides 5, pp 235-244, 1983.

Paper V — The effect of prolonged strain on serum levels of human pancreatic polypeptide (hPP) and group I pepsinogens (PGI).
O Øktedalen, P K Opstad, R Jorde and H Waldum, Scand J Gastroenterol 18, pp 663-668, 1983.

Paper VI The fasting levels and the postprandial response of gastro-entero-pancreatic hormones before and after prolonged fasting.
O Øktedalen, P K Opstad, H Waldum, R Jorde, Scand J Gastroenterol 18, pp 555-560, 1983.

Paper VII Responses of vasoactive intestinal polypeptide, secretin and human pancreatic polypeptide to glucose during fasting.
O Øktedalen, P. K Opstad, R Jorde and O B Schaffalitzky de Muckadell, Scand J Gastroenterol 19, pp 59-64, 1984.

Paper VIII The effect of physcial stress on gastric secretion and pancreatic polypeptide levels in man.
O Øktedalen, I Guldvog, P K Opstad, A Berstad, D Gedde-Dahl, R Jorde, Scand J Gastroenterol, in press.

2 METHODOLOGICAL CONSIDERATIONS

2.1 Experimental models

2.1.1 The training course

The training course is arranged once each year. It is considered an unique stress model under controlled conditions. However, the possibilities to make investigations and manipulations during the course is limited, since this is arranged by the Norwegian Military Academy for their students.

The three main stress factors during the training course are prolonged heavy physical exercise (estimated to approximately 35% of their maximal oxygen uptake), high calorie supply deficiency (daily intake of approximately 6300 kJ against a caloric combustion of 36000-43000 kJ/24 hrs), and severe sleep deprivation (about 2 hrs of sleep during 4-5 days of training). In addition, there are some psychological stress factors due to strong military discipline with occasional irrational punishments, and unpleasant environmental factors as wetness, coldness and darkness.

The calculations of the work load and the daily caloric consumption are based upon continuous heart rate registrations during previous similar training courses (19, 20). It is concluded that the subjects are exposed to extremely hard physical exercise. A calorie expenditure of nearly 40 000 kJ/24 hrs over several days has never been registrated before.

Although the course had the same training program from one year to another, it can not be excluded that there are small changes in the work load during the different courses. This mostly because there is often a shift in the commanding officer and his staff.

The sleep deprivation was obtained because of continuous simulated combat activities. Also on the basis of continuous heart rate registration, it was calculated that the average time of sleep was about 2 hrs during the whole training course (19).

To elucidate further what influence long-term heavy physical exercise, calorie supply deficiency and severe sleep deprivation separately exerts on the blood levels of the different peptides, the subjects were in papers II, III and V divided into three groups. One group had no compensation for the stress factors, another group was compensated for the calorie deficiency, and a third group was partly compensated for the sleep deprivation. The subjects that were given additional calorie supply during the course, appeared to be in calorie balance since they had no weight loss during the course. On the other hand, the other subjects lost nearly 5 kg of body weight during the course.

In paper IV and VIII the design of the stress model is in some way changed. The military tasks are more rational, there are no punishments of the subjects, the physical exercise is especially heavy for the first three days of the course, and they are allowed 4-7 hours of sleep as a total during the course. In addition, they are given more food, but are still in calorie supply deficiency since the subjects show 2-3 kg reduction in body weight during the course.

2.1.2 The fasting models

In paper VI and VII healthy subjects are exposed to a 4-5 day period of absolute fasting while performing their daily work at a research laboratorium. The subjects have free access to water, but are given nothing else except salt tablets during the experimental periods.

2.2 Biochemical analysis

2.2.1 Gastrin

Gastrin exists in blood in different molecular forms, mainly in the component III ("little-gastrin" or gastrin-17) and the component II ("big-gastrin" or gastrin-34). There are discrepancies between laboratories in the concentrations and the response patterns of gastrin. This is mostly due to the fact that antisera react with the

different molecular forms of gastrin to varying degrees (21, 22). In view of the different biological potencies of circulating G-17 and G-34 on molar base, accurate determination of the component specificity in the gastrin assay is necessary for meaningful information. In this thesis the gastrin level was measured by two different sensitive radioimmunoassay methods. In one assay, charcoal separation technique was used and the antibody reacted with the different molecular forms with equimolar potency (23). Normal serum values were found to range from 10-12 pM (I). In the other gastrin assay (IV, VIII), double-antibody separation technique was employed and the antibody showed 100% reactivity for G-17 while 29% reactivity for G-34. Normal plasma levels ranged from 35-39 pM. The fasting blood concentration of gastrin was found unchanged during the training course when measured by both radioimmunoassay methods (I, IV, VIII). This should imply that none of the two main gastrin components in the fasting blood were altered during the course. Consequently, the difference in normal range between the two assay methods could not be due to various immunopotency of the G-17 and the G-34 components in the two assays. The difference is rather explained by that the samples were measured by different radioimmunoassay methodic.

2.2.2 Human pancreatic polypeptide (hPP)

This peptide is considered simple to measure by radioimmunoassay. There appears to exist no heterogeneity of the peptide in blood, and the same standard and antibody are employed all over the world. In this thesis, hPP was measured directly in serum by two different radioimmunoassays (24, 25), using the same standard (615-1054B-200-8) and the same antibody (615-1054B-248-19), but where the separation procedures were different (polyethylene glycol 6000 and double-antibody, respectively). Different separation technique could be the reason why normal values measured by one of the assay methods (24) ranged from 9-32 pM, while with the other assay method (25) ranged from 5-9 pM (V, VIII). Both assays were highly sensitive (detecting limit of 6.4 pM and 1.5 pM, respectively), and there was no cross-reactivity for VIP, GIP, insulin, glucagon, motilin, CCK or gastrin (25).

2.2.3 Secretin

Radioimmunoassay of secretin in plasma has been difficult to perform mostly because of interfering plasma factors, and because the fasting secretin level in plasma appears to be in the low pM range. The two radioimmunoassay methods (26, 27) employed in this work are highly sensitive. Non-specific interference with the assay system by plasma factors has been abolished by different procedures. In one of the radioimmunoassay methods (27) the plasma was acidified, and the incubation was held at pH 4.0 in order to prevent a possible binding of secretin to plasma proteins. In addition, it appeared necessary to subtract the "apparent" secretin level in secretin-free plasma from that measured in the unknown samples. In the other secretin assay (26), unspecific plasma interference was avoided by ethanol extraction of the plasma samples, and by using charcoal-treated hormone-free plasma as blanks. It was especially noticed that this assay method showed no non-specific interference with glucose (26). In some of our studies (II, VII) glucose was a stimulating substrate. In both assay methods the normal fasting level was in the 2-4 pM range (II, VI).

The structure of human secretin is still unknown, and heterogeneity of secretin in plasma could explain discrepancies of secretin level found by different groups. Gel-permeation-chromatography studies (28) show that the antibody employed in the assay method of Schaffalitzky (26) detects only one component of secretin in human plasma.

The plasma level of secretin during starvation has been the object of dispute (29, 30, 31, 32). Paper VI contributes to that discussion since different fasting and postprandial secretin levels in plasma were measured in one fasting subject when employing two different RIA methods (26, 27). Differences in level has been proposed to be attributed to non-specific interference by lipolytic products in the assay system (32). However, that appears not a plausible explanation to the high secretin levels found in plasma during the training course (II, IV) or during the fasting experiments (VI, VII) when measured by the assay method of Schaffalitzky (26). Immunosorption studies in

plasma obtained during the training course revealed a decrease in levels from 12.0, 12.5, and 15.8 pM down to 0, 0.3, and 0.4 pM after immunosorption, respectively.

2.2.4 Vasoactive intestinal polypeptide (VIP)

The radioimmunoassay method of VIP employed in our study is sensitive enough to measure VIP in plasma under physiological conditions (33). The problem of non-specific interference by plasma proteins and other factors has been excluded by extraction of samples with ethanol. Furthermore, immunosorption studies in plasma obtained from the subjects of the training course showed a decrease in VIP concentrations from 20.5, 15.0 and 21.0 pM, to 5.5, 3.5 and 0 pM, after immunosorption, respectively. This appears to exclude a major non-specific interference in plasma during combined calorie deficiency, prolonged physical exercise, and sleep deprivation. Similar immunosorption results have been obtained during prolonged physical exercise employing the same radioimmunoassay method of VIP (34). It is also worth to notice that glucose did not interfere the binding of labeled VIP to the antibody (33). In two of our studies (III, VII) glucose was a stimulating substrate. It is concluded that VIP in plasma exists in only one immunoreactive form (35). Fahrenkrug and co-workers have shown by gel permeation and ion-exchange column chromatography (35) that VIP in plasma elutes in a position similar to the octacosapeptide VIP isolated by Said and Mutt from porcine intestine (36).

2.2.5 Group I pepsinogens (PGI) and pepsin

The radioimmunoassay method of PGI is run directly in serum using hormone free serum in the standards (37). The method is considered sensitive with detection limit (2.6 ng/ml) far beneath the normal level in serum, and the non-specific serum interference appears negligable.

The pepsin concentration in gastric juice was measured spectrophotometrically by a well used modified haemoglobin substrate method (38).

2.2.6 Gastric acid measurement

In paper IV the gastric juice was collected in 15 minute samples and measured for volume and acid concentration. The efficacy of the aspiration technique was not measured in that study, but studies with markers in one of the laboratories have shown that the recovery of the aspiration in an unstimulated period is 84 per cent.

In paper VIII polyethylenglycol (PEG) was used for calculating the efficiency of the gastric aspiration. Both studies (IV, VIII) showed a 3-fold increase in the basal acid output after physical stress in man.

3 GENERAL DISCUSSION

3.1 Gastrin

Gastrin was the first gastrointestinal hormone sequenced, synthetized and measured by radioimmunoassay. It is produced in G-cells mainly localized to the anteropyloric mucosa (39, 40), and has been shown to be released into the bloodstream as two major components, "big gastrin" (gastrin-34) and "little gastrin" (gastrin-17) (41). Most data reveals that this hormone, especially the gastrin-17 component, is involved in the regulation of the gastri acid secretion (42), but the role of gastrin in the peptic ulcer disease is still far from clear. In our studies the gastrin hormone can not be the stimulator for the basal hyperchlorhydria found during the stress period, since the gastrin level was unchanged. The data is significant since gastrin was determined by two radioimmunoassay methods with different affinity for the two gastrin components (23, paper IV). The fasting serum level of gastrin in duodenal ulcer patients is not different from that found in healthy man (43). Furthermore, there are conflicting reports regarding the relationship between basal serum level of gastrin and the acid secretion in healthy man, (44, 45), as well as in duodenal ulcer subjects (44, 45, 46).

The lack of change in fasting blood level of gastrin in our stress studies was a surprise particularly since the prolonged heavy exercise activated strongly the adrenergic system (47). These are conditions previously shown to stimulate the release of gastrin into the blood stream (48, 49, 50). In duodenal ulcer patients, there is even an increased sensitivity of gastrin release to adrenaline (51).

A decline in serum level of gastrin during long-lasting absolute fasting has been found (52), but that could not be confirmed in our fasting study (VI). A possible explanation for the discrepancy could be different immunological potencies of the antisera to the gastrin components in blood. Our gastrin assay (VI) showed for instance only about 30 per cent cross reactivity to the G-34 component which is the main molecular form of gastrin in blood in the fasting state (41).

Even during sham-feeding, a physiological way of vagal activation, there was no increase in the plasma gastrin level either during the stress period or in the control experiment (VIII). This accords with some previous results in healthy man (53, 54, 55). Although the gastrin level in plasma has been found elevated after sham-feeding in some duodenal ulcer patients, the rise could account for only a part of the acid response (56). There is indirect evidence that the vagus carries both excitatory and inhibitory fibres for the release of gastrin (57, 58). Our study (VIII) does not provide evidence for a change in the relationship between these two fibre types during long-term physical stress.

After giving a meal to the stressed subjects, the postprandial gastrin levels were higher than in the control condition (I). Also duodenal ulcer patients show higher postprandial gastrin response than normals (43), and this has been explained by a defect in the normal feedback mechanism in the antrum by which acidification inhibits the release of antral gastrin (59). It is an open question if that is the explanation for the high postprandial gastrin levels found during stress in our study (I). The prolonged postprandial gastrin rise in our study (I) could alternatively be caused by a delay in the gastric emptying (60). On the other hand, the prostprandial gastrin response has not been found altered after a prolonged period of physical exercise (48) which is known to decrease the gastric motility (61). The elevated levels could not be attributed to a higher activation of the adrenergic nervous system, which does not influence the gastrin release after meal stimulation (62, 63).

3.2 Human pancreatic polypeptide

The pancreatic polypeptide was discovered as an impurity of insulin (64), and was isolated as a linear polypeptide of 36 amino acid residues (65). It is derived primarily from the pancreas where it is localized to specific endocrine cell type, the PP-cell (66, 67). Its physiological role has not been fully delined, but current studies suggest that it may play a role in the modulation of pancreatic and biliary secretion (68, 69). The fasting level of human pancreatic

polypeptide (hPP) has been found to be increased by physical exercise (70,71), starvation (72,73), and by adrenergic stiumulation (74, 75). Thus, the increase in the sympathetic tone observed both during the training course (47) and during fasting (52) could explain the augmented fasting serum levels of hPP in our studies (I, V, VI, VII, VIII). On the other hand, a report indicates that the spontaneous secretion of hPP and gastric acid both are under vagal control, and found to fluctuate in parallel (76). Our finding of 2-3 fold increase in the fasting serum level of hPP could therefore imply an increase in the vagal drive during the stress situation (I, V, VIII).

Interestingly, we measured higher serum levels of hPP after modified sham feeding during the stress period than in the control experiment (VIII). This might indicate a higher vagal cholinergic (77, 78) activation by the modified sham-feeding during the stress situation. Another explanation to the augmented hPP levels after sham-feeding is an increase in the sensitivity of the PP-cell during multifactorial stress.

The hPP level in serum is highly increased after oral ingestion of a meal (75, 79, 80), but is unchanged after intravenous infusion of nutrients (81). The hPP response to a meal is biphasic, a rapid vagal cholinergic primary increase (79), and a prolonged second phase which is also dependent on cholinergic pathways (75, 80, 82). Intake of a meal during the stress period provoked an increase in the postprandial serum levels of hPP (I,V), and the integrated hPP-response was augmented (V). It is tempting to suggest that most of this effect was caused by the calorie deficiency. The postprandial output was lower in the iso-caloric subjects (V), and we found an increase in the postprandial serum levels also during absolute fasting (VI). Furthermore, a previous report (83) shows that the hPP response to a meal is unchanged after a period of total parenteral nutrition when the subjects were in calorie balance. Based on previous investigations (24, 75), it might be assumed that some change in the adrenergic mechanism is involved in the augmented postprandial hPP-release in our studies (I, V).

Oral glucose intake is only a small stimulus for release of hPP to blood (72, 84), and intravenous infusion of glucose even decreases the serum level of hPP in healthy man (72, 84). A small increase to oral, and a slight decrease to intravenous administration of glucose were also observed during a period of absolute fasting (VII). Since both stimulations were followed by hyperglycemia, it is suggested that the PP-cell in man is not directly affected by glucose either during normal conditions (85) or during absolute fasting (VII) where the glucose homeostasis is highly altered (86).

3.3 Secretin

Secretin was the first substrate considered a hormone (87). It is produced in specific endocrine cells, S-cells, localized to the duodenal and the proximal jejunal mucosa (88). For the moment, acidification of the duodenum is considered the most important stimulus for secretin release (28, 89). The duodenal pH threshold for secretin response in man is said to be 3.0 (90), but the strength of the stimulus appears to be more dependent on the amount of acid entering the duodenum (91). Accordingly, an elevated plasma level of secretin has been reported when the gastric acid secretion is enhanced as in duodenal ulcer patients (28, 92), in patients with Zollinger-Ellison syndrome (28, 92), after pentagastrin stimulation (28, 93), and at insulin-induced hypoglycemia (28). In contrast, low levels have been found when the acid delivery to the duodenum is reduced as in patients with achlorhydria (28), during aspiration of acid (28), and during treatment with histamine H_2-blockers (28).

The 3-6 fold increase in the fasting plasma level of secretin in our stress studies is much higher than the levels found during other physiological conditions employing the same radioimmunoassay method (26, 28, 94). There is reason to conclude that this stress-induced hypersecretinemia is partly due to the hyperchlorhydria found during the same period of stress (IV). Gastric aspiration as well as ingestion of cimetidine, a histamine H_2-receptor antagonist, both resulted in a decrease of the fasting plasma secretin level of approximately 50 per cent. It must be mentioned that the efficacy of the acid aspiration

was not controlled in that study, and the cimetidine group was small. These precautions can not invalidate the question of what else is the stimulus to secretin release during such a period of physical stress. The plasma secretin levels have previously been found increased both during prolonged physical exercise (34) and during prolonged fasting (30, 31, 86). In our study, the elevated plasma levels were maintained after 8 hrs of rest, and the levels were even as high in the subjects of nearly calorie balance, so it does not appear plausible that these are the stimuli to our finding of hypersecretinemia during stress.

There exists discrepancy in the results of postprandial plasma levels of secretin in man. No elevation (95, 96), sustained rises (97), a fall (98), and spike-like transient increase (28, 99, 100) has been reported. A plausible explanation to these divergent results of mean plasma level of secretin is that secretin is released intermittently when acid enters the duodenum, and with different timing from one person to another. The rapid fall in the postprandial secretin levels in plasma observed in our stress study (II), may be explained if an increase in the duodenal acidity is the main causal factor. Ingestion of a meal will have a combined buffering (91) and diluting effect on the increased gastric acid content, and the acid load delivered to the duodenum will therefore be reduced. A similar dilution effect can explain the instant fall in plasma secretin levels after an oral glucose load is given to the stressed subjects (II).

Though the postprandial secretin levels in plasma generally are low, they appear to influence the bicarbonate secretion into the duodenum (94, 101), and secretin plays probably a contributing role in the physiological regulation of the pancreatic bicarbonate secretion in man. The high plasma level of secretin in our study has possibly a key function to normalize the intraduodenal acidity when man is exposed to prolonged physical stress.

The variations in plasma secretin levels during fasting in man has been the subject of controversy (29, 30, 31, 32). Our results from the two fasting experiments (VI, VII) support the prior reports that plasma secretin is highly increased in starving man (30, 31, 86), but

this has not been confirmed by others (29, 32). The discrepancy in the results has been proposed to be attributed to plasma interference in the radioimmunoassays of secretin (32). Paper VI partly deals with that discussion, since the plasma secretin levels in one fasting subject were measured by two different RIA methods. There was a very high increase in the fasting plasma level when employing a RIA method using the procedures of plasma extraction and charcoal separation. A smaller increase was observed when a RIA method using double-antibody technique and without plasma extraction, was employed. A further discrepancy in the two sets of results was seen when the postprandial plasma levels of secretin during fasting were measured. Apparently the procedure of plasma extraction is important. See methodological considerations, secretin.

Employing the method of plasma extraction and charcoal separation technique, an instant fall in the plasma levels was found after oral as well as after intravenous glucose ingestion during fasting (VII). The variation in the plasma levels of secretin appeared to follow the blood glucose levels inversely; the lowest concentration of secretin was found to coincide with the highest glucose level, and both the blood glucose and the plasma secretin normalized simultaneously. This could give rise to the suggestion that the subsequent decline in plasma levels of secretin after glucose intake is not attributed to any effect of glucose on "luminal" receptors of the endocrine S-cells. It is rather a support for a metabolic release mechanism of this hormone during fasting.

3.4 Vasoactive intestinal polypeptide

Vasoactive intestinal polypeptide has a widespread distribution, occurring mainly in the central nervous system (102) and the digestive tract (102,103) where it possibly has a neurotransmitter function (104). It has a wide spectrum of biological activities including relaxation of vascular (105) and non-vascular (106) smooth muscles, and hypersecretion from intestinal glands (107). Concerning our studies, especial attention should be paid to its metabolic actions of stimulating the glycogenolysis (108), the gluconeogenesis (108) and

the lipolysis (109) as shown by in vitro studies. Paper VI and VII confirm the previous finding that the plasma level of VIP is enhanced during fasting (110), and paper III lend support to the reports that the plasma level is also elevated by prolonged physical exercise (34, 110). On the other hand, plasma VIP level has not been found changed during short-time, intensive physical exercise (110).

Mechanical and chemical stimulations of the jejunal mucosa have shown to evoke VIP-release to blood (111), and it is suggested that VIP may be involved in the gastrointestinal tract to facilitate the passage of material through the gut (112). On this background, it is assumed that the high plasma levels of VIP play a conceivable role in the development of diarrhoea in VIP-producing tumour patients (113).

However, such a function of VIP on the gut motility appears not to be present in our studies since the gut motility is rather known to be decreased by physical exercise (61), and since all the participants experienced a less frequency of defecation during the experimental periods than otherwise.

Peripheral plasma level of VIP is not influenced by a meal or glucose intake in healthy man (114, 115). This in spite of results from other studies which indicate a role for VIP at hyperemia of intestinal mucosa after instillation of nutrient solution in the gut (111). It was an interesting and new finding that the high plasma levels of VIP measured during the training course (III) and during the fasting experiments (VI, VII), decreased rapidly on oral ingestion of nutrients. Apparently, in one of the fasting experiments (VII) the plasma VIP levels fluctuated inversely with the blood glucose levels. This in contrast to a positive correlation between the VIP and the blood glucose levels shown after glucose loading in gastrectomi patients (116). Also in those patients the glucose metabolism was disturbed (116). During our fasting experiment (VII), we found that the augmented VIP levels in plasma were suppressed by the administration of glucose intravenously as well as orally. Similar to the secretin, this indicates that the VIP-release is not mediated by "luminal gut-factors", but is rather metabolic dependent. Altogether, our findings of high VIP levels in plasma during combined prolonged physical exercise and

starvation (III, IV), during absolute fasting (VI, VII), and the rapid return of VIP to normal plasma levels after oral as well as after intravenous nutrient ingestion (VII), support the concept that VIP is "a peptide of substrate need".

In paper III we found that VIP levels in plasma was not influenced by the hyperchlorhydria during the stress period. Although VIP is released to blood after exogenous acidification of the duodenum (117), and some studies indicate that VIP inhibits the stimulated gastric acid secretion (118), VIP has still an uncertain role in the pathogenesis of ulcer disease.

3.5 Group I pepsinogens and pepsin

Group I pepsinogens (PGI) are synthetized and stored in the chief cells of the oxyntic mucosa (119). From here it is either leaked unchanged to blood or delivered into the gastric lumen as the proteolytic enzyme of pepsin (120). Serum PGI are suggested to be a measurement of the continuous synthesis or the amount of PGI stored in the chief cells (121, 122, 123). The finding of a decrease in serum PGI during the physical stress period (V) indicates hypoactivity of the chief cell, but the cell is well preserved as the level normalized rapidly afterwards. A positive correlation is peviously reported between high gastric acid secretion and serum PGI (124). It is therefore surprising to find a decrease in serum PGI when there was a 3-fold increase in the basal acid output during a similar stress period (IV, VIII). On the other hand, Waldum et al (123) found that the serum PGI was only positively correlated to the maximal gastric acid secretion, and that was hardly influenced by the physical stress (IV, VIII). In paper V we concluded that the decline in serum PGI during stress was due to the long-term physical exercise, and that the serum PGI level normalized after 8 hours of rest. The subjects were given two to three hours of rest while being transported to the laboratories for measurements of the gastric secretion, and it remains an open question if that short period of rest was enough to obtain normal serum level of PGI after physical stress. If so, our data does not counteract that of Waldum et al (123). Furthermore, a rapid recovery

after rest would better explain why the pepsin content in the gastric juice, which is correlated to serum PGI (123), was unchanged after the stress period (VIII). On the other hand, the pepsin secretion generally follows the acid secretion, and that was highly increased in the basal state (IV, VIII). We therefore suggest that the hypersecretion of acid after physical stress is mediated through other pathways than that of pepsin release.

Recent studies have evaluated if serum PGI is a genetic marker for the predisposition to duodenal ulcer disease (125, 126, 127). More than one half of the duodenal ulcer patients have shown an elevated fasting serum PGI level (126). The stressed subjects in our studies were probably also in a disposition to develop peptic ulcer disease (128), and they showed the combination of acid hypersecretion and a decrease (probably normal levels) in serum PGI. The importance of serum PGI in evaluation of duodenal ulcer disease must therefore be handled with care, since the predisposition of developing peptic ulcer disease is often a combination of environmental and genetic factors.

3.6 Gastric acid secretion

It is widely assumed that excessive gastric acid production is an important pathogenetic factor in duodenal ulcer disease, but the pathophysiological mechanisms by which excess acid makes a hole in the duodenal mucosa, is still uncertain. Not all people with hyperacidity get ulcers, and only one third of the duodenal ulcer patients are hypersecretors, whenever they are measured at basal condition or after maximal acid stimulation (129). Most peptic ulcer patients notice exacerbations of their symptoms in connection with stressful events (130, 131) which many of them experience more frequently than healthy man (15). There are data on a relationship between stress-related anxiety and acid hypersecretion. For instance, an increase in the basal acid secretion has been reported during stressful interviews and prior to surgery in ulcer patients (132, 133), or before important school tests in healthy subjects (134). However, our reports (IV, VIII) are the first to show 3-fold increase in the basal gastric

acid output after being exposed to physical stress parameters as prolonged physical exercise, calorie supply deficiency, and severe sleep deprivation in combination.

It is well known that vagal activation stimulates the gastric acid secretion (135), and Dragstedt et al (136) introduced vagotomy as a surgical procedure to duodenal ulcer disease based upon the hypothesis of an hyperactivity of the vagus nerves in the duodenal ulcer patients. There are also some indirect evidence for an increase in the spontaneous vagal drive in the duodenal ulcer patients (137, 138). In paper VIII we have investigated the importance of the vagus nerve in the hypersecretion of gastric acid after a period of physical stress. We found 3-fold increase in the basal gastric acid output (BAO), while the acid secretion after the pure vagal stimulation of modified sham-feeding was not affected by stress when subtracted for the BAO. Furthermore, we found no change in the pepsin content of the gastric juice after stress (VIII). The gastric secretion of acid and pepsin are both strongly influenced by the vagal activity (135, 139, 140), which was considered increased after the stress when the hPP secretion was taken as a measure of the vagal tone. The dissociation of the acid and pepsin response might be taken as the result of unequal activity of the vagal fibres on the parietal cells and the chief cells. Another explanation is that the hypersecretion of acid after stress is caused by a specific hypersensitivity of the parietal cells.

In paper IV we found that the basal hyperchlorhydria was histamine dependent as the increase was prevented by the specific histamine H_2-receptor antagonist of cimetidine. There is still a question of which function histamine has in the cephalic, the gastric and the intestinal phases of acid secretion; it may be a final common mediator, or it may be a general sensitiser to other stimuli on the oxyntic cell. According to Konturek et al (141), the H_2-receptors are more important than the cholinergic receptors in the activation of the oxyntic cell during modified sham-feeding (141). It is therefore tempting to suggest that in our studies (IV, VIII) the same H_2-receptors on the parietal cell take part in the hypersecretion of acid at basal condition and in the secretion of acid after modified sham-feeding.

The hypersecretion can hardly be caused by the gastrin since the plasma concentration of that hormon was not changed by the stress (IV, VII), and not altered after modified sham-feeding (VIII). The slight hypoglycemia found (IV, VIII) is not an explanation of the hyperchlorhydria (142). During a similar stress period an increase in the sympathetic tone was found (47), but norepinephrine (143), epinephrine (144), and sympathetic stimulants (145) are all known to have an antisecretory effect on the parietal cells, and should therefore rather prevent the development of hyperchlorhydria.

Since physical exercise is found to decrease the gastric acid secretion (146), and the parietal cell shows histological signs of hypoactivity during starvation (147), the severe sleep deprivation contributes probably most to the hypersecretion of gastric acid in our model.

The gastric mucosa is thought to be most vulnerable to damage by gastric acid when there is no buffering effect of food. Our findings are of special interest in relation to the higher acid secretion between meals found in man after physical stress. There is a recent report (128) that stressful situations cause increase in the secretion of gastric acid, which, in turn, leads to the development of peptic ulcer. Therefore, precautions should be taken when man is exposed to stressful life events. Our results show that ingestion of the H_2-receptor antagonist cimetidine prevents the development of hyperchlorhydria found in man immediately after having finished a prolonged period of physical stress.

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hPP and Gastrin Response to a Liquid Meal and Oral Glucose during Prolonged Severe Exercise, Caloric Deficit, and Sleep Deprivation

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Oektedalen O, Flaten O, Opstad PK, Myren J. hPP and gastrin response to a liquid meal and oral glucose during prolonged severe exercise, caloric deficit, and sleep deprivation. Scand J Gastroenterol 1982, 17, 619–624.

Sixteen young healthy military cadets were subjected to prolonged severe exercise, caloric supply deficiency, and sleep deprivation during a 5-day ranger training course. Several cadets complained of gastric discomfort. The fasting and postprandial human pancreatic polypeptide (hPP) and gastrin levels induced by a liquid meal (no. = 9) and peroral glucose load (no. = 7) were measured during normal school activities (control) and on the third day during the course. The results showed that the fasting level of hPP was significantly increased during the course. Both during meal and glucose stimulation the hPP level during the course was significantly higher at most registrations than during control conditions. The fasting level of gastrin was not changed. The maximal level of gastrin during meal stimulation was higher during the course than during the control period. Glucose loading, on the other hand, did not change the gastrin response. The integrated response of hPP and gastrin were not changed during the course either for the liquid meal or for the peroral glucose load.

Key words: Caloric deficit; exercise, physical; gastrin; glucose, oral load; human pancreatic polypeptide (hPP); meal, liquid; radioimmunoassay; sleep, deprivation

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The gastrointestinal hormones human pancreatic polypeptide (hPP) and gastrin are released from endocrine cells localized in the pancreas and the gastric antrum, respectively (1–3). Both hormones are released after a meal (4–7) and by sympathetic activation. The serum concentration of hPP is shown to be increased during physical exercise and starvation (8–12), and Berger et al. (8, 9) found that the hPP release during exercise was mediated by a beta-adrenergic activation. Gastrin has been shown to be released by adrenaline infusion (13–17) and during prolonged physical exercise (18). Gastrin, however, is decreased during prolonged starvation (19), and no change in meal-induced postprandial gastrin levels has been found after prolonged starvation (20).

Norwegian military cadets participate every summer in a 5-day training course with severe physical exercise, caloric deficit, and sleep deprivation. During a previous, similar course, changes in the plasma concentration of several hormones were observed. The human growth hormone and adrenaline increased two- to six-fold (21), and the glucose level was significantly decreased (Opstad, unpublished observations). There is no previous report on the fasting and the postprandial blood concentration of hPP and gastrin during prolonged physical exercise combined with starvation and sleep deprivation after such a military training course.

MATERIALS AND METHODS

Subjects. Sixteen military cadets of the Norwegian Military Academy participated in a ranger training course as a part of their training program.

They were divided into two groups (group 1, no. = 9, mean age, 22.8 years; and group 2, no. = 7, mean age, 23.9 years). All were in excellent mental and physical condition with no history of gastrointestinal disease.

Diet. The daily food intake for each cadet consisted of about 95 g of protein, 65 g of fat, and 125 g of carbohydrates, representing 6300kJ or about one sixth of their total caloric expenditure. The water intake was unlimited.

Training course. The course took place in a forest area at an altitude of about 500 m and lasted from Monday morning (day 1) until the following Friday afternoon (day 5). The subjects had a mean work load of 35% of their maximal oxygen uptake, and the caloric expenditure was estimated to be about 36,000kJ/24 h (22, 23). Owing to continuous simulated combat activities, the subjects had only 1–2 h of sleep totally during the course, which fits with conclusions drawn from continuous pulse registration during previous similar courses (22, 23). Thus, the multifactorial strain was composed of prolonged physical exercise (about 35% of their maximal O_2 uptake), caloric deficit (given one sixth of the expenditure), and sleep deprivation (2 h of sleep during 5 days).

Tests and methods. All stimulation tests were performed during a rest period in the morning after an overnight fast. A standardized liquid meal consisting of 80 g Biosorbin (Pfrimmer) dissolved in 200 ml of water and 200 ml of milk (totally containing about 16 g of protein and about 2300kJ) was given to group 1 on day 3 during the course and about 6 months after the course (control). In group 2, 100 g of glucose dissolved in 100 ml of water was given perorally on day 3 during the course and about 1 week before the course (control). Blood from an antecubital vein was drawn before (−15), during (0), and 15, 30, 45, 60, and 90 min after start of the peroral stimulation. Blood was prepared for serum and kept frozen (−40°C) until analysis. Gastrin was determined with a radioimmunoassay method (24) having a sensitivity of 6 pg/ml, a within-assay precision of 8%–12%, and a between-assay precision of 11%. The serum level of hPP was determined with a radioimmunoassay method (25) having a sensitivity (detection limit) of 6.4 pM, a within-assay precision of 11.5%, and a between-assay precision of 13%.

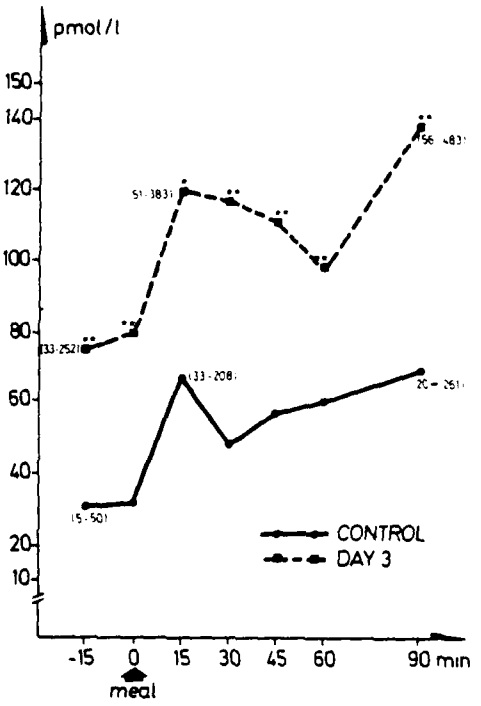

Fig. 1. Fasting and postprandial hPP levels induced by a liquid meal given perorally in a control period (control) and on the third day of a military training course with prolonged physical exercise, caloric deficit, and sleep deprivation (day 3). Median values with total range in parentheses. * $p < 0.05$ compared with control; ** $p < 0.005$ compared with control.

Statistics. The results are given as median and with total range in parentheses. Variations in results within the same group were estimated by the Wilcoxon signed rank test. The integrated peptide response was calculated by trapeze integration from zero to the 90-min sample, with the pre-stimulatory peptide concentration subtracted.

RESULTS

hPP

The fasting median level of hPP increased about 230% ($p < 0.05$) in group 1 and about 310% ($p < 0.05$) in group 2 during the course (Figs. 1

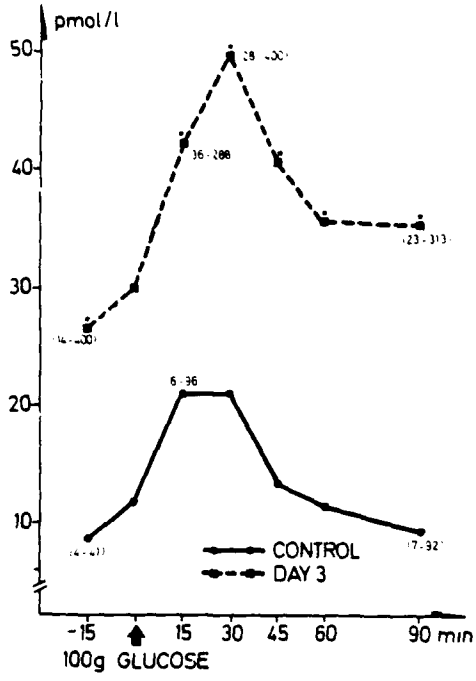

Fig. 2. Fasting and postprandial hPP levels induced by a peroral glucose load (100 g) given in a control period (control) and on day 3 during the combat course. Median values with total range in parentheses. * $p < 0.05$ compared with control.

and 2). The hPP level increased rapidly after meal stimulation and more slowly during glucose loading.

The absolute increase (pmol/l) after glucose and meal stimulation was similar during the control period and during the course. Compared with the control, the postprandial hPP levels were therefore significantly increased ($p < 0.05$) at most registrations during the course (Figs. 1 and 2). Because of great individual variance in response (Table I) the integrated hPP response (IhPPR) was not changed either for the liquid meal or for the peroral glucose load during the course.

Gastrin

Compared with control the fasting level of gastrin was slightly but not significantly increased during the course (Figs. 3 and 4). The gastrin levels at 30 and 45 min after meal stimulation were significantly increased during the course ($p < 0.02$, Fig. 3). The gastrin level remained elevated but was not increased compared with control 90 min after meal stimulation.

The postprandial gastrin levels after glucose loading were not significantly changed during the course. The integrated gastrin response (IGR) to both the liquid meal and the peroral glucose load was not significantly changed during the course (Table I).

DISCUSSION

The present study has shown that the fasting serum concentration of hPP and the postprandial serum concentration of hPP and gastrin are increased in healthy man exposed to prolonged multifactorial strain. The increased fasting level of hPP observed during the course is consistent with the prior finding of elevated hPP both during physical exercise (8–11, 26) and starvation (10). Recent investigations have shown that the serum concentration of hPP in man is influenced by adrenergic modulation both before (8, 9, 25) and after oral glucose (25). An increase in the fasting level of adrenaline has previously been observed

Table I. The integrated gastrin response (IGR) and integrated hPP response (IhPPR) induced by a liquid meal and peroral glucose load given during a control period (control) and during a military training course (course) with prolonged physical exercise, caloric deficit, and sleep deprivation*

	IGR		IhPPR	
	Meal	Glucose	Meal	Glucose
Control	413(188–1140)	278(15–720)	2565(−491 to 14363)	300(−1913 to 4166)
Course	533(60–2085)	330(248–585)	2318(−5025 to 24008)	1148(−120 to 3645)

* Median values expressed in pmol·min/l with total range in parentheses.

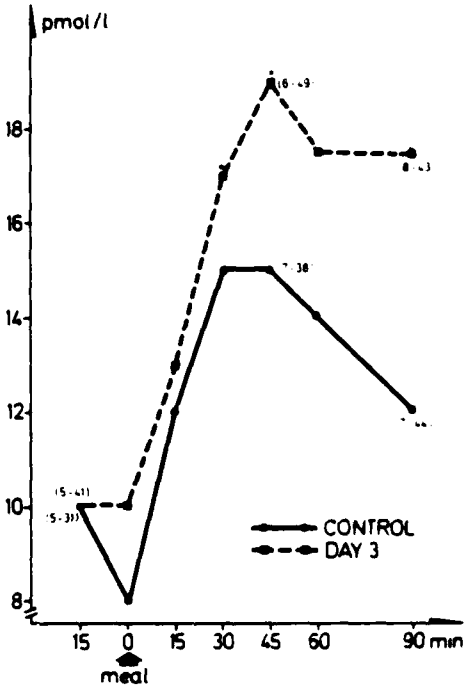

Fig. 3. Fasting and postprandial gastrin levels induced by a liquid meal given perorally in a control period (control) and on the third day of a military training course with prolonged physical exercise, caloric deficit, and sleep deprivation (day 3). Median values with total range in parentheses. * $p < 0.05$ compared with control.

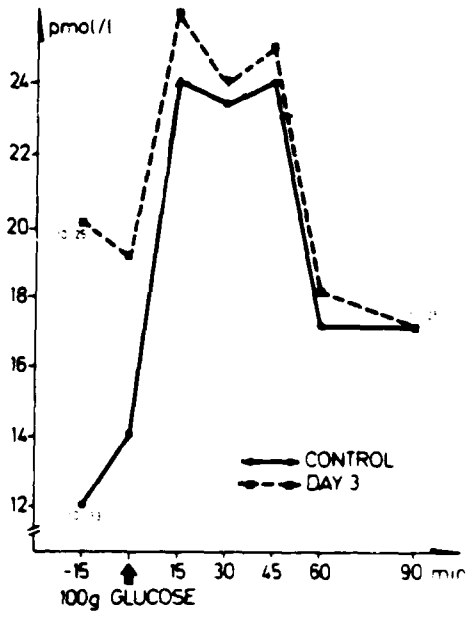

Fig. 4. Fasting and postprandial gastrin levels induced by a peroral glucose load (100 g) given in a control period (control) and on day 3 during the combat course. Median values with total range in parentheses.

during a similar military training course (21). This alteration in the sympathetic tone might explain the increased fasting and postprandial serum concentrations of hPP during the course. In addition, the release of hPP is under strong vagal control (27–29). Hypoglycaemia is a potent vagal-dependent stimulus of the hPP release (10, 30). During a previous, similar training course the fasting blood glucose level was decreased (Opstad, unpublished observations). The increased fasting serum concentration of hPP might thus be related to the fasting hypoglycaemia observed during the course. However, this does not explain the increased serum concentrations of hPP after oral glucose and liquid meal.

The fasting gastrin level has previously been found to increase after prolonged physical exercise (18), but to decrease during starvation (19). The previously observed increase in the fasting level of adrenaline and decrease in the fasting level of glucose during a similar military training course are expected to enhance the gastrin release in man (13, 17, 31). In addition, the previously registered fivefold increase in human growth hormone and the significant increase in cortisol (21) are assumed to promote gastrin release, as shown in animals (32, 33). Even in the presence of somatostatin-stimulating factors, the fasting serum concentration of gastrin was not changed during the course, which, in addition, should indicate some other inhibitory mechanisms. In our experiment the maximal concentration of gastrin induced by the liquid meal was increased during the course. Previously, the meal-induced gastrin release has not been found to be influenced either by physical exercise (18) or by prolonged fasting (20). Perhaps the prolonged multifactorial strain including sleep deprivation could promote the postprandial

gastrin release. It is tempting to speculate whether the increased sensitivity of the G-cells to a liquid meal observed in our study could be a mechanism similar to that reported in duodenal ulcer patients, in whom the postprandial serum concentration of gastrin has also been found increased (5, 34–37).

Previous investigation has shown that the meal-induced gastrin response is, in contrast to the fasting level of gastrin, not mediated by beta-adrenergic receptors but by receptors that recognize proteins and digested products (5). Our results indicate that these receptors might be influenced by prolonged multifactorial strain.

In conclusion, we have found that the fasting serum concentration of hPP in healthy man is elevated during prolonged, multi-factorial strain and that this increase might be explained by the increased sympathetic tone or the hypoglycaemia observed during the strain. The increased release of both hPP and gastrin after a liquid meal indicates an increased sensitivity of the PP-cell and the G-cell during such strain.

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Secretin—a new stress hormone?

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Summary

The plasma concentration of secretin was measured during a 5-day military training course comprising prolonged physical exercise (35% of max O_2 uptake), severe caloric deficiency (approx. 35700 kJ/24 h) and sleep deprivation (only 2 h of sleep as a total during 5 days). 24 subjects were divided into 3 groups, one group was compensated for the caloric deficiency and another group was partly compensated for the sleep deprivation. The results showed that the fasting plasma secretin increased 3–6-fold (from 1.8–3.7 to 13.3–19.1 pmol/l) during the course with small differences in increase between the groups. Ingestion of a mixed meal reduced the fasting plasma secretin by about 50% during the course, while oral glucose reduced the plasma secretin to the concentrations found in the control experiment.

The study shows that plasma secretin is increased when man is exposed to prolonged multifactorial stress. Additional food or sleep appears to have small influence on the fasting plasma secretin, but after giving a meal or oral glucose solution the plasma secretin decreases rapidly.

physical exercise; caloric deficiency; sleep deprivation; secretin

Introduction

Secretin is a peptide hormone considered to play a major role in regulation of pancreatic secretion of bicarbonate. Secretin is released into the blood stream after intraduodenal instillation of acid [17] or bile [14]. So far the delivery of gastric acid to the duodenum is the only well established physiological stimulus for release of secretin. The physiological metabolic importance of secretin during caloric deficiency has been discussed. Prolonged starvation has been reported to have no effect [6] or to elicit increased concentrations of secretin in plasma [7,8,12]. In vitro experiments have shown that secretin stimulates lipolysis [10,15], glycogenolysis [13]

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and gluconeogenesis [13]. Furthermore, elevated concentrations of secretin in plasma have been observed after prolonged physical exercise [9].

The present work was undertaken to study the metabolic importance of secretin during a training period, combining heavy physical exercise with caloric deficiency and severe sleep deprivation.

Materials and Methods

24 military cadets of the Norwegian Military Academy participated in a training course which lasted from Monday (day 1) until the following Friday evening (day 5). The subjects were randomly divided into three groups (group I, $n = 7$, median age 23 years, range 22–24 years; group II, $n = 8$, median age 24 years, range 22–26 years; and group III, $n = 9$, median age 23 years, range 21–25 years). All cadets were exposed to prolonged heavy physical exercise previously estimated to be about 35% of their maximal oxygen uptake [1,21]. Because of continuous simulated combat activities the subjects in groups I and II had only 1–2 h of sleep in total during the course, while the subjects in group III regularly got 3 h of additional sleep each night. The subjects of groups I and III were exposed to high caloric deficiency, while the cadets in group II were almost isocaloric.

Daily basic food intake for all men consisted of about 95 g protein, 65 g fat and 125 g carbohydrate, representing approximately 6300 kJ. In addition, each cadet in group II was given a special compound diet containing about 105 g protein, 125 g fat and 1230 g carbohydrate. This diet was given as soup, orange juice, cocoa and milk shake and represented about 26900 kJ/24 h. The caloric expenditure during the course was estimated to range from 36100 to 42800 kJ/24 h [1,21]. Thus, groups I and III had a caloric deficiency of 29400–36500 kJ/24 h, whilst those in group II had minimal caloric deficiency. The cadets of group II showed no significant loss of body weight, whilst each cadet in groups I and III lost about 4.5 kg bodyweight during the course.

Blood for secretin determination was collected after an overnight fast immediately before the course started (day 1), every morning during the course and after 8 h rest (day 6). The effect of nutrients was studied by giving a test meal and a 100 g oral glucose load after an overnight fast on separate days during the course. The test meal (2200kJ) which consisted of one egg, two pieces of cheese-sandwiches and 200 ml of milk was given on day 3 during the course (day 3) and in a control period (control) 8 weeks after the course. The glucose load (100 g glucose in 400 ml water) was given on day 5 and day 6 during the course and in a control period (control) 8 weeks after the course. The meals and glucose loads were consumed within 3 min. Blood was drawn from an antecubital vein 15 min before (−15) and 15, 30, 60, 90 and 120 min after the meal was given. On day 5 and in the control period blood was drawn 15 min before (−15) and 30, 60, 90 and 120 min after the start of the glucose load, while on day 6 samples were obtained at −15, 30 and 90 min. Samples for secretin determinations were collected into ice-chilled glass tubes containing heparin and aprotinin (Trasylol®, 500 K.I.U. aprotinin per ml blood) and left on ice until

centrifugation. Aliquots of plasma were stored at −20°C until radioimmunoassay for secretin [16].

The results are presented as median with total range in brackets. Variations in results within the same group were estimated according to the Wilcoxon matched pairs signed-rank test whilst differences between groups were assessed by the Mann–Whitney *U*-test.

The level of statistical significance was 0.05.

Results

Compared to day 1 there was a 3–6-fold increase in the fasting plasma secretin in all the groups during the course (Table I). In group I the increase was highest on days 2 and 5 ($P < 0.05$) whilst in groups II and III the increase was highest on day 2 ($P < 0.05$). 8 h after the course was finished (day 6) the fasting plasma secretin was still 3–5-fold elevated (Table I) but in all the groups the increase was smaller than on days 2 and 5 during the course ($P < 0.05$). The increase in fasting plasma concentration of secretin did not differ between the groups apart from a larger increase in group I than in group III on days 5 and 6 ($P < 0.05$).

After ingestion of a mixed meal the plasma secretin declined in all the groups within the first 30 min. A nadir of 45% of fasting value was reached after 30–60 min but the secretin levels did not reach the postprandial values obtained in the control experiment, except at 120 min in group II (Fig. 1). There was a tendency of increase in postprandial plasma secretin concentration later than 60 min.

Plasma secretin decreased rapidly in all the groups after glucose loading on day 5 during the course, reached the plasma concentration found in the control experiment

TABLE I

Fasting plasma concentration of secretin (pmol/l) before (day 1), during a prolonged training course and 8 h after the course was completed (day 6)

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Group I	2.5 (0.9–5.1)	18.3 * (12.8–31.1)	11.0 * (6.9–13.7)	10.8 * (8.8–16.0)	19.1 * (9.6–25.0)	11.5 * (6.7–17.7)
Group II	3.3 (0–4.8)	13.3 * (5.0–38.2)	9.7 * (2.1–10.7)	9.8 * (5.8–19.2)	12.0 * (5.3–23.0)	8.7 * (3.2–13.4)
Group III	1.8 (1.0–3.4)	15.2 * (7.2–30.0)	9.5 * (4.2–20.4)	8.3 * (4.1–10.0)	9.7 * (4.0–24.2)	5.2 ** (0.2–13.0)

The subjects in group I were exposed to prolonged physical exercise, severe caloric deficiency and sleep deprivation. Groups II and III were similar to group I except that group II was kept almost isocaloric and group III got 3 h additional sleep each night during the course. Values are median with total range in parentheses.

* $P < 0.01$ compared to day 1.

** $P < 0.02$ compared to day 1.

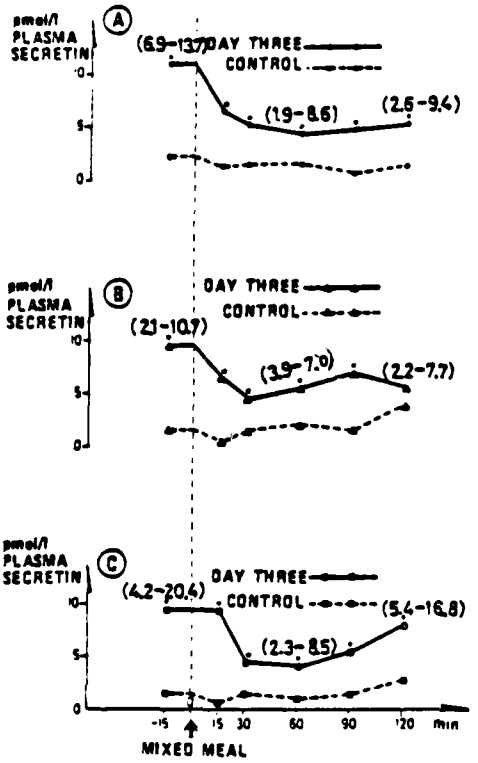

Fig. 1. The meal-induced changes in plasma concentration of secretin (pmol/l) in a control period (control) and on day 3 during a prolonged training course. The subjects in group I (A), group II (B) and group III (C) were exposed to the stress factors as described in Materials and Methods. Values are median with total range in parentheses. * $P < 0.01$ compared to control.

within 60 min and remained at this level throughout the experiment (Fig. 2). Glucose loading on day 6 resulted in a similar decline in plasma concentration of secretin as found on day 5 (Fig. 2).

Discussion

The present study showed that the plasma concentration of secretin increased 3–6-fold when man was exposed to prolonged physical exercise, severe caloric deficiency and sleep deprivation. The secretin concentration was still elevated after 8 h rest. The high plasma level of secretin decreased after a mixed meal, but did not reach the plasma concentrations found after a meal in the control experiment.

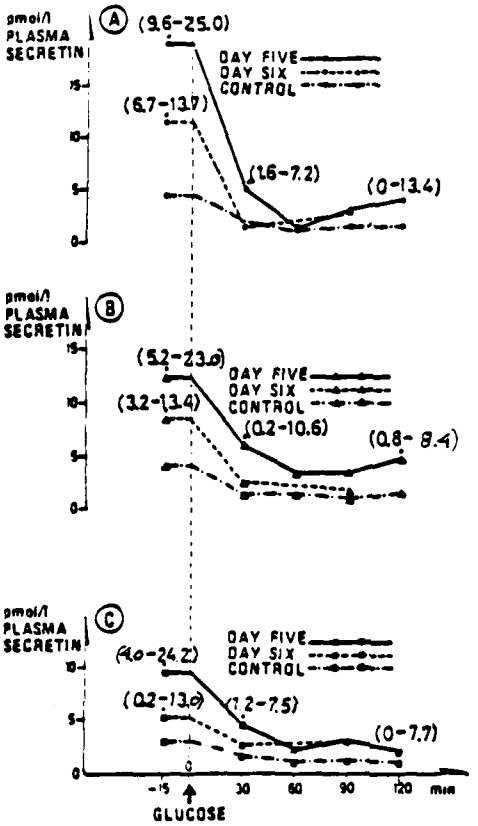

Fig. 2. The glucose-induced changes in plasma concentration of secretin (pmol/l) in a control period (control), on day 5 of a prolonged training course and 8 h after the training course was completed (day 6). Group I (A), group II (B) and group III (C) were exposed to the stress factors as described in Materials and Methods. Values are median with total range in parentheses. * $P < 0.05$ compared to control.

However, after ingestion of a hypertonic glucose solution the plasma concentration of secretin decreased rapidly to control values both during the course (day 5) and after the course was completed (day 6). Caloric compensation did not influence the concentration of secretin in plasma (group II versus group I), whilst additional sleep (group III versus group I) caused a smaller increase (Table I).

Intraduodenal acidification is the only well established stimulus for secretin release [2,3,17]. Previous studies have shown that the emptying of gastric acid into the duodenum regulates the plasma concentration of secretin in man [4,17]. The secretin levels found in the present study are considerably higher than the values measured during more physiological conditions employing the same radioimmunoas-

say [5,17–19]. The cause of these high values is not immediately intelligible. In view of the lipolytic [10,15], gluconeogenetic [13] and glycogenolytic [13] actions of secretin demonstrated in vitro, it has been speculated that secretin has metabolic importance during periods of negative caloric balance. This hypothesis was supported by the findings of Henry et al. [7,8] who reported elevated concentrations of secretin in plasma after prolonged starvation, and these high concentrations were suppressed by refeeding. However, in our investigation caloric compensation did not influence the secretin concentration in plasma, therefore starvation does not seem to be the stimulus per se.

Also physical exercise of shorter duration [9] has previously been found to elicit increased concentrations of secretin in plasma. However, physical activity is not likely to be the stimulus for the observed plasma secretin concentrations since our tests were performed in a rest period, and since the fasting plasma secretin level was still 3–5-fold elevated after 8 h rest (day 6). In addition, the plasma secretin concentration decreased rapidly after ingestion of a meal or a glucose solution. The increased secretin levels could be explained by decreased elimination through the kidneys. Previous observations indicate that renal function is reduced during hard training (P.K. Opstad, unpublished results). However, the rapid decline after ingestion of a meal or glucose solution cannot be explained by this mechanism.

We thus speculate that increased acidity of duodenal contents is the causal factor for the elevated plasma concentration of secretin found in our study. The reduction in plasma concentration after ingestion of a meal or a glucose solution is presumably caused by effects that reduce the rate of acid delivered into the duodenum [19]. This reduction is accomplished either through the buffering and dilution of gastric acid by the meal [19], or through dilution of the gastric acid by the glucose solution, in combination with a delay in gastric emptying because of hyperglycemia [11] that follows the meal- and glucose-stimulations. Previously, a similar postprandial secretin response has been found in patients with Zollinger–Ellison syndrome and in duodenal ulcer patients showing gastric acid hypersecretion [20]. In our experiment we observed a smaller decrease after giving the meal than after ingestion of the glucose solution during the course (Figs. 1 and 2). This could in part be explained by the larger volume of the glucose solution compared to the meal and hence a greater dilution of gastric acid. In addition, food in contrast to oral glucose, stimulates gastric acid secretion.

In conclusion, we have found a 3–6-fold increase in fasting plasma concentration of secretin when man is exposed to prolonged stress. We propose that the possible role for secretin during this condition is to protect the duodenal mucosa against damage due to increased acidity of duodenal contents.

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Plasma Concentration of Vasoactive Intestinal Polypeptide during Prolonged Physical Exercise, Calorie Supply Deficiency, and Sleep Deprivation

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Øktedalen O, Opstad PK, Fahrenkrug J, Fonnum F. Plasma concentration of vasoactive intestinal polypeptide during prolonged physical exercise, calorie supply deficiency, and sleep deprivation. Scand J Gastroenterol 1983, 18, 1057–1062.

Twenty-four military cadets went through a 5-day period of heavy physical exercise (35% of max O_2 uptake), severe calorie supply deficiency (about 36,000 kJ/24 h), and sleep deprivation (2 h of sleep as a total during 5 days). Some cadets compensated for the caloric deficiency, whereas others partly compensated for the sleep deprivation. Fasting and meal- and glucose-induced changes in the plasma concentration of vasoactive intestinal polypeptide (VIP) were measured on separate days during the course and 8 h after the course was finished (day 6). Fasting plasma concentration of VIP increased two- to five-fold during the course, with the highest increase on day 2. The calorie-compensated subjects showed a smaller increase than those who did not receive any calorie or sleep compensation. Intake of a meal or glucose solution lowered the VIP concentration in plasma within 30–60 min to the concentrations found in the control experiments performed several weeks after the course. The results indicate a role of VIP as 'a polypeptide of substrate need'.

Key words: Caloric deficiency; physical exercise; sleep deprivation; vasoactive intestinal polypeptide

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Vasoactive intestinal polypeptide (VIP), a highly basic octacosapeptide originally isolated from porcine small intestine (1), is widely distributed in neurons throughout the body (2). There is growing evidence that VIP may be a neurotransmitter mediating several functions, like secretion and relaxation of vascular and non-vascular smooth muscle (3).

Plasma VIP concentration has previously been found to increase during prolonged fasting (4, 5) and prolonged physical exercise (5, 6). This together with its metabolic actions, including stimulation of lipolysis (7), glycogenolysis (8), and gluconeogenesis (8), indicates that VIP might have a physiological metabolic function during negative caloric balance. It was therefore considered of interest to measure fasting and nutrient-induced changes in plasma VIP concentration during a 5-day strain period in which the subjects went through prolonged physical exercise combined with severe calorie supply deficiency and sleep deprivation.

SUBJECTS AND METHODS

Twenty-four military cadets of the Norwegian Military Academy participated in a training course as a part of their obligatory military training program lasting from Monday (day 1) until the following Friday (day 5). They were randomly divided into three groups (group one, no. = 7, median age 23 years, range 22–24 years; group two, no. = 8, median age 24 years, range 22–26 years; and group three, no. = 9, median age 23

years, range 21–25 years). All cadets were exposed to prolonged physical exercise, previously estimated to be a mean of 35% of their maximal oxygen uptake (9, 10). The subjects of groups one and two had only 1–2 h of sleep as a total during the course, whereas the subjects of group three in addition had 3 h of sleep every night during the course. The subjects of groups one and three were exposed to great calorie supply deficiency, whereas the cadets of group two were almost iso-caloric.

The daily basic food intake of all cadets consisted of 95 g protein, 65 g fat, and 125 g carbohydrate, representing approximately 6300 kJ. In addition, each cadet in the iso-caloric group (group two) was given a special compound diet containing 105 g protein, 125 g fat, and 1230 g carbohydrate. This diet was given as soup, orange juice, cocoa, and milk shake and represented approximately 27,000 kJ/24 h.

The caloric expenditure during the course was estimated to range from about 36,000 to 43,000 kJ/24 h (9, 10). Thus, the subjects in groups one and three had a large caloric deficiency of about 30,000–37,000 kJ/24 h, whereas those in group two were nearly in caloric balance. The cadets of group two showed no significant loss of body weight, whereas the cadets in groups one and three lost about 4.5 kg (median; range, 2.5–6.0 kg) during the course.

Blood for determination of fasting plasma VIP concentration was drawn in the morning immediately before the start of the course (day 1), every morning during the course (day 2 until day 5), and in the morning of day 6 after 8 h of sleep (day 6). Furthermore, the nutrient-induced changes in the plasma concentration of VIP were examined during the prolonged strain period. A mixed meal or 100 g glucose solution was ingested after an overnight fast on separate days during the course. The mixed meal (2200 kJ), which consisted of an egg, two cheese sandwiches, and 200 ml of milk, was given on day 3 during the course (day 3) and in a control period (control) 8 weeks after the course was finished. The glucose load (100 g glucose in 400 ml water) was given on days 5 and 6 during the course (day 5 and day 6) and in a control period (control) 8 weeks after the course was finished. The meals and glucose loads were consumed within 3 min. Blood was drawn from an antecubital vein 15 min before (−15) and 15, 30, 60, 90, and 120 min after the meal was given. On day 5 and in the control period blood was drawn 15 min before (−15) and 30, 60, 90, and 120 min after the start of the glucose load, whereas on day 6 samples were obtained at −15, 30, and 90 min. Samples were collected in ice-chilled glass tubes containing heparin and aprotinin (Trasylol®; 500 KIU aprotinin/ml blood) and left on ice until centrifugation. Aliquots of plasma were stored at −20°C until measurement of VIP by radioimmunoassay (11).

The results are presented as medians, with total range in parentheses. Variations in results within the same group were estimated by Wilcoxon's matched-pairs signed-rank test, whereas differences between groups were assessed by the Wilcoxon–Mann–Whitney test. $p < 0.05$ was considered statistically significant.

RESULTS

Compared with the pre-course value (day 1) the fasting plasma concentration of VIP increased two- to five-fold during the course, with the highest increase on day 2 (Table I). After 8 h of rest (day 6) the concentration of VIP was still 2- to 2.5-fold elevated, although significantly smaller than the increase observed on days 2 and 5. Comparison between the groups showed that the increase was significantly higher in group one than in group two on days 3, 5, and 6 and greater than in group three on day 6.

After intake of the meal and the glucose solution the concentration of VIP decreased rapidly in all the experimental groups, whereas no change was observed in the control experiments. During the meal stimulation the VIP concentration in plasma reached the same level as in the control experiment within 30–50 min (Fig. 1). There was a tendency towards an increase in the postprandial VIP concentration in plasma later than 90 min.

During the glucose stimulation on day 5 the VIP concentration in plasma returned to the level found in the control experiment after 30 min, and

Table I. Fasting plasma concentration of VIP (pmol/l) before (day 1), during (days 2–5), and 8 h after (day 6) a prolonged training course*

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Group one	10.0 (7.0–13.0)†	34.0 (17.0–60.0)	31.0 (13.5–62.0)	18.0 (15.5–56.0)	29.0 (23.5–72.5)	25.0 (20.0–80.0)
Group two	7.0 (6.0–9.0)	27.0 (2.5–48.5)	16.0 (8.5–30.5)	18.5 (6.0–24.0)	19.0 (11.5–55.5)	13.0 (6.5–22.5)
Group three	7.0 (3.5–13.5)	33.5 (15.0–43.0)	16.5 (11.0–39.0)	15.0 (7.0–28.5)	20.0 (12.5–39.0)	15.0 (5.5–16.5)

* The subjects in group one were exposed to prolonged physical exercise, severe calorie deficiency, and sleep deprivation. Groups two and three were similar to group one except that group two was almost iso-caloric and group three got 3 h additional sleep every night during the course.

† Median with total range in parentheses.

the concentration remained at this level throughout the experiment. Glucose loading on day 6 resulted in a similar decline in plasma concentration of VIP as found on day 5 (Fig. 2).

DISCUSSION

Our investigation has shown that the fasting plasma concentration of VIP is two- to five-fold increased when man is exposed to a prolonged period of multifactorial strain comprising heavy physical exercise, calorie supply deficiency, and severe sleep deprivation. The plasma level was partly reduced in the subjects who received additional calories (group two) but not significantly reduced in the subjects receiving additional sleep (group three). During the course, but not in the control studies, the plasma level of VIP was rapidly reduced by the supply of calories both in the form of meal and glucose solution. The high VIP level could not be due to plasma artefacts, since immunosorption studies exclude interference with strain-induced plasma products in the radioimmunoassay of VIP (Fahrenkrug, personal communication). Previous investigations have shown that the VIP concentration in plasma increases during long-term physical exercise (5, 6) and after a prolonged fasting period (4, 5). However, there are no earlier reports on the VIP level in human plasma during a 5-day period of heavy physical exercise combined with calorie supply deficiency and sleep deprivation.

In our study we suggest that both the starvation and the heavy physical exercise contribute to the rise in plasma concentration of VIP. Thus there was smaller increase in the fasting plasma concentration of VIP both when additional food was given (Table I, group two versus group one), and after 8 h of rest (Table I, day 6). Among the wide range of biological actions of VIP, the stimulation of the lipolysis (7), the glycogenolysis (8), and the gluconeogenesis (8) appear most relevant for the present study, in which all the subjects were exposed to long-term, heavy physical exercise and in which there was increased demand for fat mobilization owing to the great physical activity or to the starvation. During a previous similar training course the lipolysis was found highly

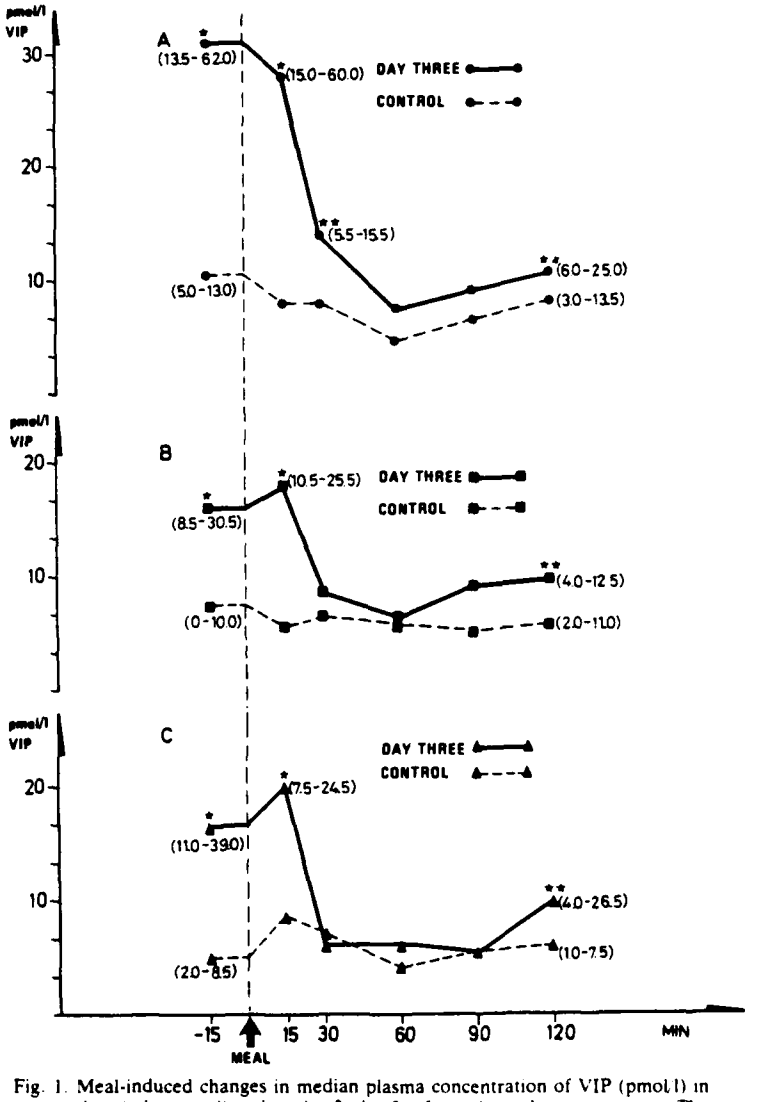

Fig. 1. Meal-induced changes in median plasma concentration of VIP (pmol/l) in a control period (control) and on day 3 (day 3) of a prolonged training course. The subjects in group one (A), group two (B), and group three (C) were exposed to the strain factors as described in Subjects and Methods. Total range in parentheses. *$P < 0.005$ compared with control; **$P < 0.05$ compared with control.

increased (12), and our finding of a high plasma concentration of VIP may indicate some physiological metabolic function of VIP. This is further supported by the rapid decline in the plasma concentration of VIP after oral ingestion of nutrients. Results from another experiment with prolonged physical exercise have shown that the administration of glucose contributes to the metabolic substrate utilization within 15 min and that the glucose intake induces an increase in the

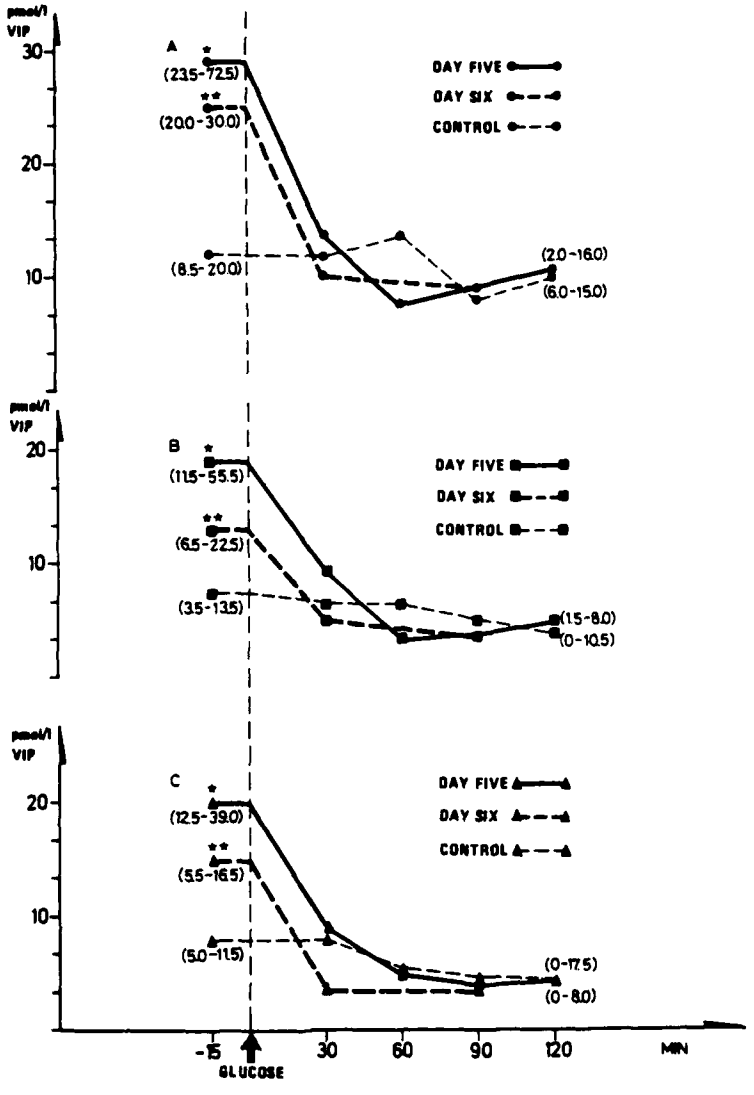

Fig. 2. Glucose-induced changes in median plasma concentration of VIP (pmol/l) in a control period (control), on day 5 of a prolonged training course (day 5), and 8 h after the training course was finished (day 6). Group one (A), group two (B), and group three (C) were exposed to the strain factors as described in Subjects and Methods. Total range in parentheses. $^{*}P < 0.01$ compared with control; $^{**}P < 0.02$ compared with control.

total carbohydrate metabolism with a corresponding decrease in the lipid mobilization (13). The nutrient-induced decrease in VIP level in plasma during the strain period might therefore fluctuate synchronously with a decrease in the lipolysis after nutrient intake.

The still high post-strain plasma level of VIP on day 6 was most probably due to the calorie

deficiency of the subjects. Even the subjects of the high-calorie group (group two) showed an accumulating calorie deficiency on day 6 of the course. On this day, as on day 5 during the course, the glucose ingestion was followed by a decrease in the plasma level of VIP. Similar to the plasma concentration of VIP in our investigation, the blood level of the lipolytic product of free fatty acids (FFA) has previously been shown to decrease rapidly on refeeding after starvation (14). This lends further support to a metabolic release mechanism for VIP during a prolonged period of strain and to the concept that VIP is 'a polypeptide of substrate need'. A possible metabolic role for VIP should also be seen in the light of probably higher VIP concentration in portal blood (15, 16) than in peripheral blood, from which our samples have been taken.

The liver appears to be involved in the elimination of splanchnically released VIP (17). The liver function, however, is suggested not to be changed during the training course (Øktedalen et al., unpublished), and consequently the rise in the fasting plasma concentration of VIP should not be due to a decrease in the hepatic elimination. Furthermore, the suppressive effect of a meal or glucose solution cannot be explained by this mechanism.

In conclusion, we have found that the fasting plasma concentration of VIP increases considerably in man exposed to a prolonged period of physical exercise, caloric deficiency, and sleep deprivation. The plasma concentration returns rapidly to control level after oral ingestion of a meal or glucose solution. The results indicate a metabolic release mechanism of VIP during prolonged multifactorial strain.

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Basal hyperchlorhydria and its relation to the plasma concentrations of secretin, vasoactive intestinal polypeptide (VIP) and gastrin during prolonged strain

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Summary

Twenty young men divided into two groups participated in a five day training course with prolonged and heavy physical exercise, calorie supply deficiency and severe sleep deprivation. Basal acid output (BAO) was measured immediately after the course in seven of ten subjects who were given placebo tablets (placebo group) and in four of ten subjects who had a daily intake of 1 g cimetidine (cimetidine-group) during the course. Median BAO increased 3-fold in the placebo subjects (from 2.7 mmol/h to 8.2 mmol/h) but showed no increase in the cimetidine treated subjects. The median fasting plasma concentrations of secretin increased 2–8-fold during the course. Gastric suction for 1 h or ingestion of cimetidine reduced the plasma concentration of secretin by approx. 50%. Vasoactive intestinal polypeptide (VIP) increased 2-fold and was not influenced by reduction of gastric acid. The placebo group showed a small increase ($P < 0.05$) in plasma concentration of gastrin on day two during the course.

The study shows a marked hyperchlorhydria which partly explains the fasting hypersecretinemia found during prolonged strain. This strain-induced hyperchlorhydria could be abolished by treatment with the selective H_2-receptor antagonist cimetidine.

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calorie deficiency; gastric acid secretion; gastrin; physical exercise; secretin; sleep deprivation; VIP

Introduction

Both physical and psychological stress have been shown to cause gastric erosions in animals [1,2] and are considered to be possible etiological factors of peptic ulcer disease in man [3]. The gastric acid secretion has been found to increase after severe trauma and injuries [4,5] and during emotional stress [6,7]. This is in contrast to physical exercise which appears to diminish the gastric acid secretion [8,9]. During a previous long-term training course combining heavy physical exercise with calorie supply deficiency and sleep deprivation, a 3–6-fold increase in the plasma concentrations of secretin [10] and vasoactive intestinal polypeptide (VIP) [11] was found. Duodenal acidification is the only known physiological stimulant for secretin release [12], and exogenous duodenal acidification has also been shown to elevate the plasma concentration of VIP [13]. Furthermore, a feedback mechanism exists by which acidification of the antrum inhibits the release of gastrin to blood [14]. A recent report indicates increment of gastrin in blood during long-term exercise [15]. However, during a previous training course the blood concentration of gastrin was found unchanged [23], which might be attributed to gastric hyperchlorhydria. Therefore the present study was undertaken to measure the gastric acid secretion and its relationship to the blood concentrations of secretin, VIP and gastrin during a similar long-term training period.

Materials and Methods

Twenty military cadets (between 20 and 30 years of age) participated in a prolonged training course as a part of their military training program. The course lasted from Monday night (day one) until the following Friday (day five). Subjects were randomly divided into two groups. From the evening of the second day the subjects of one group (cimetidine group, $n = 10$) received 1 g of cimetidine per day (200 mg three times daily and 400 mg at night). The other group (placebo group, $n = 10$) were given placebo tablets. Heart rate registrations during previous courses have shown a calorie expenditure from 36 000 kJ/24 h to 43 000 kJ/24 h and an average work load of 35% of maximal oxygen uptake [17,18]. In this study the majority of heavy physical exercise took place during the first three days. Due to continuous activities the subjects had only 6–7 h of sleep as a total during the course.

The daily food intake varied on the different days during the course. The subjects consumed k-ration (10 000 kJ) the first day, a warm meal (3400 kJ) in the evening of the second day, 6 slices of bread (2800 kJ) in addition to a warm meal (3800 kJ) in the evening of the third day, and late on the fourth day they consumed half a cooked

chicken (4400 kJ). Intake of water was ad libitum. Due to the heavy physical exercise the subjects were undoubtably in calorie deficiency and showed a 3 kg reduction in body weight. Thus the multifactorial strain was composed of prolonged and heavy physical exercise, calorie deficiency and severe sleep deprivation.

Blood for determination of secretin, vasoactive intestinal polypeptide (VIP), gastrin, glucose and creatinine was drawn after 12 h fast in a rest period during the course and in control periods 8 weeks later. Blood samples were drawn in the afternoon on day 2 and day 4 and in the morning on day 5 during the course. The same schedule of sampling was followed in the control experiments. Blood was obtained $2\frac{1}{2}$ h after intake of cimetidine or placebo tablets. In seven of the subjects of the placebo group the basal gastric acid secretion and the pentagastrin stimulated secretion of gastric acid were measured. Because of limited laboratory capacity the basal gastric acid secretion was measured in only four of the subjects of the cimetidine group.

Measurements of acid production took place immediately after the course, $2\frac{1}{2}$ h after the last intake of cimetidine or placebo tablets. The subjects were fasted for 12 h and abstinent from water for 3 h. In control experiments performed eight weeks later, the subjects had no prior ingestion of tablets. Gastric juice was collected by a Levine tube fluoroscopically-controlled in position and intermittent suction was applied. The stomach was emptied as completely as possible during a 10-min period and the aspirate was discarded. Gastric secretion was thereafter collected in 15-min portions for 1 h unstimulated and, concerning the seven subjects of the placebo group, 1 h pentagastrin-stimulated (pentagastrin subcutaneously, 6 μg/kg body weight). Acid concentration was estimated by titration to pH 7.4 using a semi-automatic titremeter (Radiometer, Copenhagen). Blood samples were drawn during acid measurements of the seven subjects of the placebo group and were obtained before and after 1 h aspiration of gastric juice. Samples for determination of secretin and VIP were collected in ice-chilled glass tubes containing heparin and aprotinin (Trasylol® 500 KIU aprotinin per ml blood), while samples for gastrin were collected in glass tubes containing EDTA and aprotinin. Samples were left on ice until centrifugation. Blood for serum was allowed to clot before centrifugation. Aliquots of plasma and serum were stored at $-20°C$. Radioimmunoassays were employed for determinations of secretin [19], VIP [20] and gastrin (Cambridge Nuclear). The gastrin assay had a sensitivity of 5 pg/ml and a specific reactivity of 100% with G-17, 29% with G-34 and 0.1% with secretin. Serum glucose was determined by hexokinase method (Boehringer, Mannheim, F.R.G.). Serum creatinine was measured according to Chasson et al. [21] modified by Tagerson and Rebel [22] by adding EDTA to the creatinine recipient solution.

The results are presented as median with total range in parentheses. Variations in results within the same group were estimated according to the Wilcoxon signed-rank test while differences between groups were assessed by Wilcoxon rank sum test. $P < 0.05$ was considered statistically significant.

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Results

Basal gastric acid secretion (Fig. 1)

The subjects of the placebo group showed that during the course the volume of gastric juice was 180% and the concentration of gastric acid 170% of the respective measurements in the control period. Thus basal acid output (BAO) increased 3-fold (range 2–17) in the placebo group during the course. In the cimetidine group the concentration of gastric acid and the basal acid output appeared even lower during the course than in the control experiment in which no tablets were given.

Maximal gastric acid secretion (Fig. 1)

Maximal gastric acid secretion during the course showed a small but insignificant increase in the volume and acidity of the gastric juice, while the acid output was increased ($P < 0.05$) compared to the control experiment.

Plasma concentration of secretin (Table I)

Secretin concentration in plasma showed 2–5-fold increase ($P < 0.001$) during the

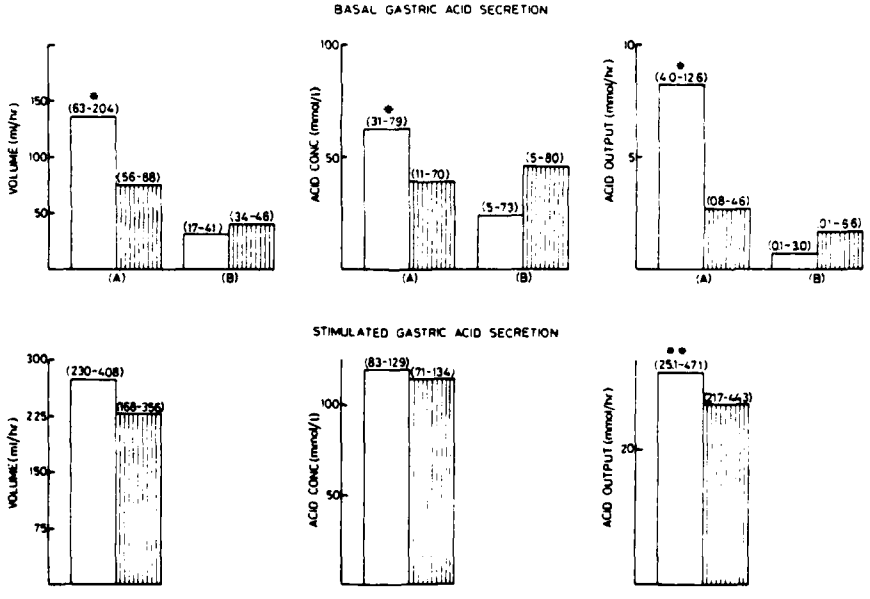

Fig. 1. Upper panel: The basal acid secretion was measured after a five-day stress period (open column) and in control experiment (hatched column). (A) Seven subjects took placebo tablets during the stress period while in (B) four subjects had a daily intake of 1 g of cimetidine during the stress period.
Lower panel: Pentagastrin (6 μg/kg body weight) stimulated secretion of gastric acid in seven subjects who had a daily intake of placebo tablets during a 5-day stress period. Experiments were performed at the end of the stress period (open column) and under control conditions 8 weeks later (hatched column).
* $P < 0.01$ compared to control period. ** $P < 0.05$ compared to control period.

TABLE I

Variations in gastrointestinal peptides during prolonged strain in man

		Control period		Stress period		
		Afternoon	Morning	Day 2	Day 4	Day 5
Secretin	placebo	3.2	2.5	8.5 *	7.6 *	12.3 *
	group	(0–7.6)	(0–3.6)	(4.2–26.8)	(2.8–19.2)	(6.0–20.4)
	cimetidine	1.8	0.5	14.0 *	6.5 **	5.8 *
	group	(0.4–9.0)	(0–4.0)	(4.6–39.0)	(2.0–12.2)	(3.8–14.6)
VIP	placebo	8.1	6.9	16.0 **	14.1 **	13.5 **
	group	(2.9–14.0)	(1.0–11.0)	(11.7–21.8)	(7.9–21.2)	(3.1–20.9)
	cimetidine	10.6	9.6	17.6 **	16.6 **	17.2 **
	group	(3.2–15.6)	(3.6–13.8)	(12.0–21.8)	(10.8–51.4)	(5.4–37.0)
Gastrin	placebo	45	46	51 ***	46	47
	group	(36–58)	(35–51)	(37–84)	(39–64)	(34–53)
	cimetidine	47	43	42	45	44
	group	(35–56)	(13–83)	(24–51)	(27–73)	(32–49)
Glucose	placebo	4.4	4.8	4.4	4.5	4.0 ***
	group	(4.1–4.8)	(4.1–5.5)	(3.4–5.1)	(3.1–5.4)	(3.2–5.1)
	cimetidine	4.5	5.4	4.9	4.5	4.4
	group	(3.4–3.0)	(4.0–7.2)	(3.2–5.6)	(3.0–6.3)	(2.5–4.9)
Creatinine	placebo	86	92	103 ***	97 ***	96
	group	(72–100)	(72–106)	(84–123)	(8.4–109)	(78–107)
	cimetidine	98	99	110 ***	108 ***	107
	group	(81–111)	(84–122)	(104–150)	(81–129)	(80–118)

Median concentrations (total range in brackets) of secretin (pmol/l), vasoactive intestinal polypeptide (VIP) (pmol/l), gastrin (pg/ml) in plasma, and concentrations of glucose (mmol/l) and creatinine (μmol/l) in serum are shown on separate days during a prolonged training course and in control periods. The cimetidine group ($n = 10$) had a daily intake of 1 g cimetidine, while the placebo group ($n = 9$) took placebo tablets on day 4 and day 5 of the course

* $P < 0.001$ compared to control period.

** $P < 0.005$ compared to control period.

*** $P < 0.05$ compared to control period.

course but there was no significant change in concentration from one day to another during the course in the subjects of the placebo group. In the subjects of the cimetidine group an 8-fold increase was found in the plasma concentration on day 2. After ingestion of cimetidine the plasma concentration decreased by 55–60% ($P < 0.01$) on day four and day five when compared to day two during the course (cimetidine group, Table I). The secretin level in plasma was higher in the cimetidine group than in the placebo group on day 2, while there was no difference in plasma levels between the two groups on day 4 and day 5 during the course.

Gastric aspiration for 1 h diminished secretin in plasma by approx. 55% ($P < 0.01$) but did not suppress the concentration to values found in the control experiment (Fig. 2). During the control experiment the decrease in plasma secretin after gastric aspiration was not statistically significant.

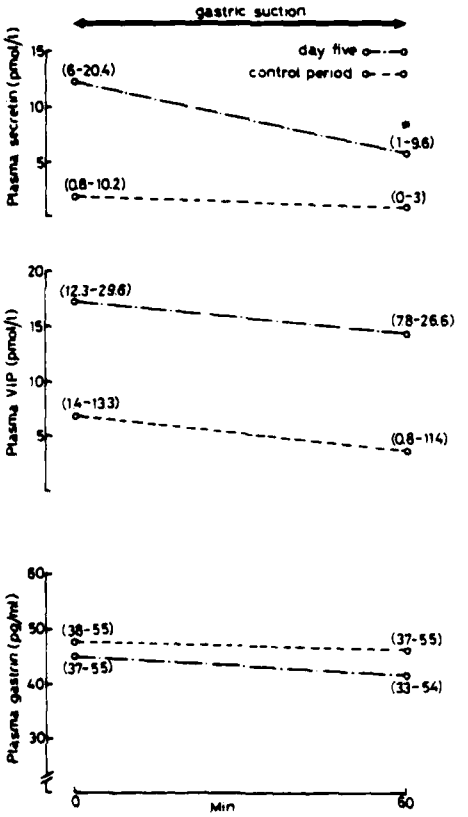

Fig. 2. The effect of gastric aspiration on the plasma concentrations of secretin, vasoactive intestinal polypeptide (VIP) and gastrin performed after a 5-day stress period (– · – · –) and under control conditions 8 weeks later (- - - - - -). Median with total range in parentheses. * $P < 0.01$ compared to presuction value.

Plasma concentration of VIP (Table I)

This increased approximately 2-fold on day 2 and remained at this level throughout the course. Neither intake of cimetidine (Table I) nor gastric aspiration for 1 h (Fig. 2) changed the plasma VIP concentrations during the course.

Plasma concentration of gastrin (Table I)

Gastrin concentration showed no change during the course apart from a slight increase in the placebo group on day 2 ($P < 0.05$).

Serum concentration of glucose (Table I)

Glucose was unchanged apart from a small decrease ($P < 0.02$) in the placebo group on day 5 during the course.

Serum concentration of creatinine (Table I)

Creatinine showed values within the normal level but with great individual variations. The concentration increased slightly in both groups ($P < 0.05$) on day 2 and day 4 during the course. The cimetidine group showed higher ($P < 0.05$) concentrations than the placebo group measured on day 2 and in the control experiments.

Discussion

Our study has shown that basal gastric acid secretion is considerably increased when man is exposed to a prolonged period of multifactorial stress comprising long-term, heavy physical exercise, calorie supply deficiency and sleep deprivation. Interestingly, the rise in the volume of acid and the rise in the concentration of acid contributed approximately equally to the overall increment in acid secretion. Although the efficiency of the aspiration was not evaluated, these findings seem to exclude reflex from the duodenal content. There are no previous reports on acid secretion from the stomach during this kind of activity, but the result accords with the augmented secretion found in man during emotional stress [6,7] while it is at difference with the decrease in secretion noticed during physical exercise [8,9]. The reason for the increment in gastric acid secretion during prolonged, multifactorial stress seems difficult to explain. However, a stress-induced increase in the tonic vagal activity which is assumed to take part in the spontaneous acid secretion [14] might be responsible for the increase. Indirect support for this assumption is that the fasting blood concentration of human pancreatic polypeptide (hPP) has been found to be increased 2–3-fold during a similar training course [23], and Schwartz et al. [16] found a positive correlation between fluctuation in spontaneous acid secretion and fluctuation in plasma PP concentration which he suggested was vagal mediated. Ingestion of cimetidine, a histamine H_2-receptor antagonist, prevented any increase in basal acid secretion. This indicates that the stimulus for the augmented acid secretion is histamine dependent and that prolonged stress in man might be accompanied by an increase in the release of histamine from gastric mucosa as

interpreted in duodenal ulcer patients [24]. Furthermore, it has been shown that the mucosal histamine release is vagal mediated both in duodenal ulcer patients [25] and in stressed rats [26]. However, the blocking effect of cimetidine on the acid secretion could also reflect the increase in the sensitivity of the histamine H_2-receptor which has recently been reported in training rats [27] and suggested in duodenal ulcer patients [28]. The augmented secretion of gastric acid is hardly mediated by gastrin since that hormone was unchanged on day 5 even after the acid was aspirated.

The neural and hormonal stimulatory mechanisms on the gastric secretory cells are closely interwoven. Vagal stimulation of gastric secretory cells has been shown to potentiate the gastric secretory response to exogenous gastrin in animals [29], but this effect does not appear important in our investigation since the pentagastrin-stimulated secretion of gastric acid was only slightly augmented from the measurements in the control period. Neither is the small decrease in fasting blood glucose found immediately prior to the acid measurements a reasonable explanation for the increase in secretion of gastric acid [30]. Several lines of evidence indicate that the acid secretion is inhibited by norepinephrine [31] as well as by epinephrine [32] and stimulation of the sympathetic nervous system [33]. It is therefore noteworthy to find the gastric acid secretion highly augmented in spite of the increase in the sympathetic tone previously recorded during a similar training course [34].

The concentration of secretin in plasma is regulated physiologically by endogenously produced acid [12]. In our study the increase in fasting plasma secretin concentration was in part explained by the basal hyperchlorhydria. Gastric aspiration for 1 h reduced the fasting secretin concentration in plasma by 55% and the same reduction was obtained after ingestion of cimetidine which prevented any increase in gastric acid secretion. This contrasts to the finding in the subjects of the placebo group who showed no change in the plasma secretin concentration on the different days during the course. The plasma secretin concentration on day 2 during the course was higher in the cimetidine group than in the placebo group. This could possibly explain the lack of change in plasma concentration between the two groups on day 4 and day 5 during the course.

Though the fasting hyperchlorhydria contributes to approximately one half of the hypersecretinemia found during prolonged strain, it is still uncertain what the other stimulus might be. Prior studies report an increase in plasma secretin concentration during prolonged physical exercise [35] and during starvation [36,37]. However, calorie supply and 8 h rest did not influence the 5-fold increase of secretin in plasma previously found during strain [10]. The hypersecretinemia can hardly be explained by a decrease in the elimination of secretin by the kidneys since the blood concentration of creatinine indicated a normal renal function. Ingestion of cimetidine induced a slight increase in the serum concentration of creatinine which is in agreement with a previous finding [38]. Control experiments demonstrated that all measurable secretin in plasma was removed after immunosorption by specific secretin antiserum (5591-7) [19]. Consequently the increased concentration of secretin immunoreactivity in plasma is not caused by 'non-specific' plasma artifacts.

The concentration of VIP in plasma showed a smaller increase than reported from a previous training course [11]. VIP has been considered a "polypeptide of substrate

need", and the difference in calorie supply with a smaller calorie deficiency in this study as opposed to the prior study might explain the difference in the plasma concentration. The plasma concentration of VIP was not influenced by endogenous acid.

In conclusion, the study has shown that multifactorial stress is followed by increased secretion of gastric acid and that the augmented production of gastric acid can in part explain the hypersecretinemia found in man during prolonged stress.

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The Effect of Prolonged Strain on Serum Levels of Human Pancreatic Polypeptide and Group I Pepsinogens

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Øktedalen O, Opstad PK, Jorde R, Waldum H. The effect of prolonged strain on serum levels of human pancreatic polypeptide and group I pepsinogens. Scand J Gastroenterol 1983, 18, 663–668.

Twenty-four young male subjects participated in a 5-day training course with long-term physical exercise (35% of Vo_{2max}), calorie supply deficiency (intake of approximately 6300 kJ 24 h, against a combustion of approximately 40,000 kJ 24 h), and severe sleep deprivation (2 h of sleep as a total during 5 days). The subjects were divided into three groups; one group (no. = 7) had no compensation for the stress factors, another group (no. = 8) compensated for the calorie deficiency, whereas a third group (no. = 9) partly compensated for the sleep deprivation. Fasting serum concentration of human pancreatic polypeptide (hPP) and group I pepsinogens (PGI) were measured immediately before the course, every morning during the course, and 8 h after the course. In addition, the serum response of hPP to a test meal was measured on day 3 during the course and in a control study performed 8 weeks later. The fasting serum concentration of hPP showed a two- to three-fold increase during the course in the low-caloric but not in the iso-caloric subjects. The serum concentration of hPP was decreased to pre-course levels after 8 h of rest. The postprandial hPP response was elevated in all the subjects during the course, with a greater increase in the low-caloric subjects than in the subjects with calorie balance. Serum concentration of PGI was 10–30% decreased during the course, and the levels were normalized after 8 h of rest after the course. The study shows that the function of the hPP cell and the chief cell is influenced by prolonged, multifactorial strain. Especially calorie deficiency appears to affect the release of hPP.

Key words: Exercise, physical; human pancreatic polypeptide; group I pepsinogens; sleep, deprivation; starvation.

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The human pancreatic polypeptide (hPP) is considered a gastrointestinal hormone, but its physiological role has not yet been established. It is well documented that the secretion of hPP is stimulated by food ingestion (1, 2). However, studies have also shown a rise of hPP in blood in the inverse situation of prolonged fasting (3, 4). In fasting experiments plasma hPP shows circadian variations, with higher concentration in the evening than in the morning (3, 4). This overnight fall in plasma hPP level might be associated with sleep (3).

Furthermore, there are reports of augmented blood levels of hPP during long-term physical exercise (4–6), and Berger et al. (7, 8) found that the hPP release during exercise was caused by beta-adrenergic activation.

During a long-term training course with prolonged heavy physical exercise, calorie supply deficiency, and severe sleep deprivation and during which the adrenaline concentration in blood increased two- to six-fold (9), we found a 200–300% increase in the fasting blood concentration of hPP (10). In addition, during a similar course

we also recorded a threefold increase in the basal gastric acid secretion (11). This gastric hyperchlorhydria might be associated with an augmented blood level of group I pepsinogens (PGI) since there exists a positive correlation between high gastric H^+ secretion and serum PGI (12). Starvation has also been shown to increase the serum PGI (13). In view of these effects on hPP and PGI in blood, this study was designed to investigate what influence long-term physical exercise, calorie supply deficiency, and severe sleep deprivation separately exert on the fasting serum level of hPP and PGI and on the postprandial hPP release during a similar prolonged training course.

MATERIALS AND METHODS

Twenty-four military cadets of the Norwegian Military Academy participated in a 5-day training course that lasted from Monday (day 1) until the following Friday evening (day 5). The subjects were randomly divided into three groups (group one, no. = 7, median age 23 years, range 22–24 years; group two, no. = 8, median age 24 years, range 22–26 years; and group three, no. = 9, median age 23 years, range 21–25 years). All the cadets were in excellent mental and physical condition with no history of gastrointestinal disease. They were exposed to prolonged heavy physical exercise, previously estimated to be approximately 35% of their maximal oxygen uptake (14, 15). The subjects of groups one and two had only 1–2 h of sleep as a total during the course (14, 15), whereas the subjects of group three regularly got 3 h of additional sleep each night. The subjects of groups one and three were exposed to large calorie deficiency, whereas the cadets of group two were almost in calorie balance. The daily basic food intake for each cadet consisted of 95 g protein, 65 g fat, and 125 g carbohydrate, corresponding to approximately 6300 kJ. The subjects had free access to water. In addition, each cadet in group two was given a special compound diet containing 105 g protein, 125 g fat, and 1230 g carbohydrate. This diet was given as soup, orange juice, cocoa and milk shake and represented approximately 27,000 kJ/24 h.

The calorie expenditure during the course was estimated to range from 36,100 to 42,800 kJ 24 h (14, 15). Thus groups one and three had a large calorie deficiency of 29,400–36,500 kJ/24 h, whereas those in group two had minimal calorie deficiency. The cadets of group two showed no significant weight loss, while each cadet in groups one and three had a median weight loss of 4.5 kg during the course.

Blood for determination of hPP and PGI was drawn after an overnight fast immediately before the course started (day 1), every morning during the course, and finally after 8 h of rest after the course (day 6). The effect of a test meal on the hPP release was studied on day 3 during the course (day 3) and in a control period (control) 8 weeks after the course. The test meal (2200 kJ) consisted of one egg, two cheese sandwiches, and 200 ml of milk and was consumed within 3 min. Blood was drawn from an indwelling cannula 15 min before (−15) and 15, 30, 60, 90, and 120 min after starting ingestion of the meal. Blood was allowed to clot at room temperature before centrifugation. Aliquots of serum were stored at −20°C until radioimmunoassay of hPP (16) and PGI (17).

Statistics

The results are presented as medians, with interquartile range in parentheses. Variations in results within the same group were estimated by the Wilcoxon matched-pairs signed-rank test, whereas differences between groups were assessed by the Mann-Whitney U test. The integrated hPP response was calculated by trapeze integration with the pre-stimulatory concentration subtracted. p values less than 0.05 were considered significant.

RESULTS

Human pancreatic polypeptide

The fasting serum concentration of hPP was greatly increased during the course. The highest increase was observed in groups one and three on day 2 (240% increase in group one and 280% in group 3; 130% increase in group two) with a

Table I. The fasting serum concentration of human pancreatic polypeptide (hPP, pmol/l) and group I pepsinogens (PGI, μg/ml) immediately before (day 1), during (day 2 until day 5), and 8 h after (day 6) a prolonged training course. The subjects of group one were exposed to prolonged, heavy physical exercise, calorie supply deficiency, and severe sleep deprivation. Groups two and three were similar to group one except that the subjects of group two were nearly in calorie balance and that the subjects of group three got 3 h additional sleep every night during the course. Median values with interquartile range in parentheses

		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
hPP	Group one	9 (5–11)	20* (17–35)	9 (7–19)	14* (8–27)	13 (9–19)	7 (7–12)
	Group two	9 (6–12)	11* (8–18)	6 (2–6)	7 (0–14)	9 (0–10)	3 (0–7)
	Group three	7 (6–23)	20* (14–41)	10 (5–21)	19* (9–23)	15 (11–20)	8 (6–14)
PGI	Group one	128.7 (100.2–141.8)	87.7* (73.8–108.0)	98.2* (83.1–134.3)	99.8* (71.9–148.5)	113.9 (99.3–163.7)	139.7 (136.0–175.8)
	Group two	140.2 (114.6–162.8)	126.0* (87.4–146.0)	128.0* (115.2–130.1)	108.6* (91.4–130.1)	112.3* (90.0–119.5)	155.1 (149.9–189.4)
	Group three	109.5 (104.6–157.9)	90.5* (75.0–107.9)	92.3* (73.9–116.9)	87.1* (68.5–137.3)	93.0* (66.3–137.7)	110.9 (84.3–157.6)

* $P < 0.05$ compared with day 1.

smaller increase later during the course (Table I). The iso-caloric group (group two) showed no increase in fasting serum concentration except on day 2 of the course (Table I). The serum concentration was decreased to pre-course levels after the cadets were given an 8-h rest at the end of the course (day 6, Table I). There was greater absolute increase in the serum concentration of groups one and three compared with the values in group two on days 4 and 5 during the course.

Ingestion of a meal induced a rapid and large release of hPP both during the course and in the control experiment (Fig. 1). All the groups showed higher postprandial serum concentrations of hPP during the course than in the control experiment (Fig. 1). For all the groups the integrated hPP response (ihPPR) to the meal was elevated during the course compared with control, and the response was greater ($p < 0.03$) in groups one and three than in group two.

Group I pepsinogens

The fasting serum concentration of PGI showed a significant decrease of 10–30% during the course (Table I). After 8 h of rest (day 6) the serum concentration reached the pre-course levels. There was no difference in change of levels between the groups.

DISCUSSION

This study has shown a two- to three-fold increase of fasting hPP concentration in serum and a small decline in serum PGI during long-term strain including starvation in man. The fasting serum concentration of hPP was only slightly increased when the subjects were in calorie balance, and both serum hPP and serum PGI returned to pre-course values after 8 h of rest. Our hPP results accord with previous findings during physical exercise (4–8), during fasting (3, 4, 13), and during a similar training course (10). It is evident that both physical exercise and starvation raised the fasting hPP level in our study, since the concentration normalized after 8 h of rest and since a greater increase was found in the low-caloric groups than in the iso-caloric group. Recent investigations have shown that the secretion of hPP is augmented by adrenergic activation (18), which is greatly increased during this kind of training course (9). This might indicate that the increase in fasting serum concentration of hPP in our study is caused by an alteration in the sympathetic tone. However, small differences were found in the plasma level of adrenaline between the food-deprived and the iso-caloric subjects during a similar training course (Opstad et al.

unpublished observation). Schwartz et al. (19) have shown that the basal hPP secretion is regulated mainly by tonic vagal activity. Furthermore, they also found covariation between the gastric acid secretion and the blood concentration of hPP, which they proposed was due to variations in the vagal activity. In a similar training course we found a threefold increase in the basal gastric acid secretion without any hypergastrinemia (11), indicating an increase in the vagal tone. It is therefore tempting to suggest that the increment in serum hPP concentration in our study is vagally mediated.

Ingestion of the meal provoked an augmented integrated hPP response (ihPPR) during the strain, with higher postprandial output in the low-caloric subjects than in the iso-caloric subjects. The release of hPP after a meal is highly vagal-cholinergic-dependent (2, 20). During a previous similar training course elevated postprandial serum levels of hPP were also reported, but ihPRR was not altered from control experiment (10). It was recently shown that subjects with elevated basal concentrations of hPP in blood elicit a smaller hPP response to further vagal stimulation than subjects with low basal hPP concentration (21). Therefore, the discrepancy in the ihPPR result might be explained by the difference in the serum level of hPP before the meal ingestion, since this level was increased threefold in the former (10) but was unchanged in this study. Moreover, the change in responsiveness to a solid meal ingested during strain may be at variance with that to a liquid meal that was given during the previous training course (10). Previous reports indicate that the major part of the hPP response to eating is caused by the presence of food in the gastrointestinal tract and that chemical receptors within the stomach (20), the duodenum (22, 23), and the intestine (23) play an important role in the prolonged second phase of the postprandial hPP release. One might assume that the decrease in gut motility occurring during exercise (24) would prolong the time of contact of nutrient stimuli with the gastric and intestinal mucosa, resulting in a stronger signal for PP release. However, this assumption does not explain why the change in response is greater in the low-caloric subjects than in the subjects in calorie balance. The blood concentration of hPP has been shown to rise both during fasting (3, 4) and feeding (4, 20). It remains speculative whether these stimuli together potentiate the activity of the PP cell.

Serum PGI reflects the gastric H^+ secretion (25) and has been found to be increased in Zollinger-Ellison (12) and duodenal ulcer patients (12) showing increased gastric acid secretion. Despite the previous finding of a threefold increase in the basal gastric acid secretion during a similar training course, the serum PGI concentration in our study was rather decreased throughout the experiment.

It is reasonable to suggest that the prolonged strenuous exercise accounts for the decrease in serum PGI in our study, because all the subjects were exposed to long-term physical exercise, because there was no difference of change in serum PGI between the groups, and because the serum PGI returned to the pre-course value after 8 h of rest. It is interesting that the calorie balance did not exert any influence on the serum PGI concentration in this experiment, although a slight increase in the serum concentration has been reported during prolonged fasting (13). The reason for this decline in serum PGI during prolonged multifactorial strain is difficult to explain. It can hardly be ascribed to the gut-hormonal profiles during prolonged strain (11), since this should rather enhance the serum concentration of PGI. The results indicate hypoactivity of the chief cells during strain, but without any damage of the cells themselves, as in gastritis (26).

In conclusion, the study has shown that both the fasting and the food-stimulated release of hPP in serum are greatly increased in man exposed to prolonged, multifactorial strain. Further, a decrease in the serum PGI concentration was found, even though a recent report indicates gastric hypersecretion during this kind of strain.

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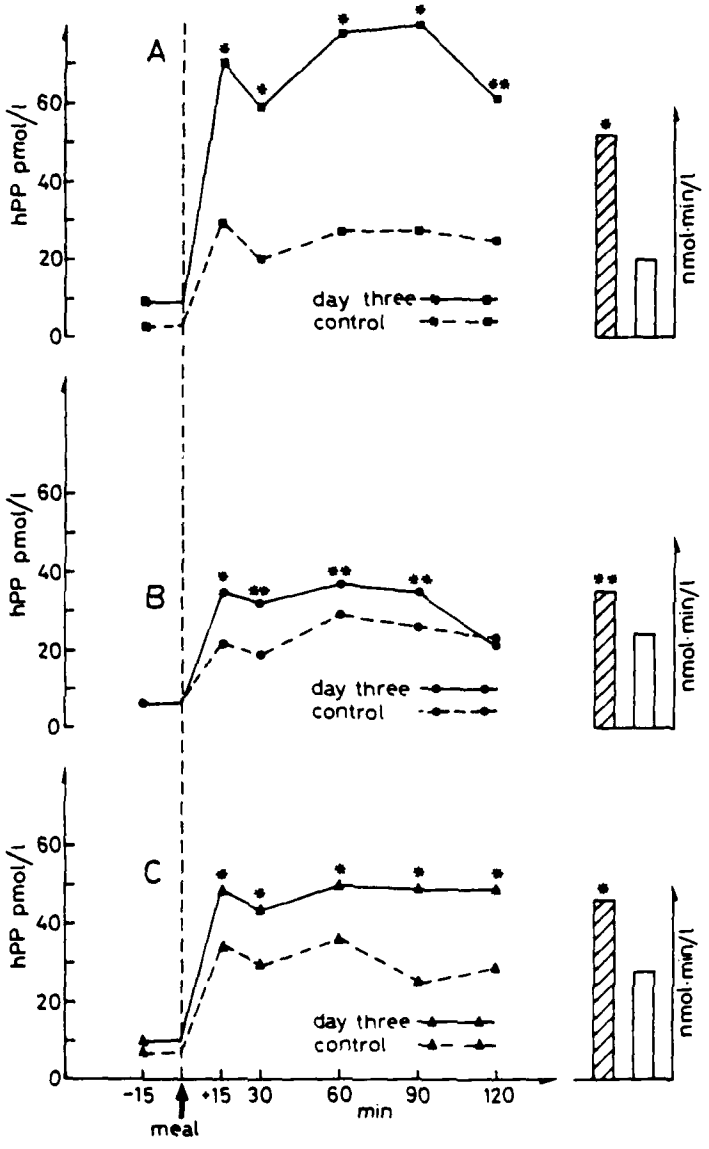

Fig. 1. *Left:* The release of human pancreatic polypeptide (hPP) after food stimulation in a control experiment (control) and on day 3 (day 3) of a prolonged training course. The subjects of group one (A), group two (B), and group three (C) were exposed to the stress factors as shown in Table I. *Right* The integrated postprandial response of hPP (ihPPR) measured over 120 min in a control experiment (open column) and on day 3 of a training course (hatched column). Median values. *$P < 0.005$ compared with control; **$P < 0.05$ compared with control

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The Fasting Levels and the Postprandial Response of Gastroenteropancreatic Hormones before and after Prolonged Fasting

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The effect of a prolonged 5-day fast on the blood concentrations of vasoactive intestinal polypeptide (VIP), secretin, human pancreatic polypeptide (hPP), gastrin, and group I pepsinogens (PG I) was studied in 11 healthy subjects. During the fast there was a marked increase in the concentrations of VIP, secretin, and hPP, whereas the rise in the concentrations of gastrin and PG I was less pronounced. Refeeding suppressed the increased concentration of VIP and caused elevated postprandial concentrations of secretin and hPP, whereas starvation did not influence the postprandial release of gastrin and PG I. The study shows that prolonged starvation has a pronounced effect on gut endocrine responses.

Key words: Fast; gastrin; glucose; meal; pancreatic polypeptide (PP); pepsinogen I; secretin; vasoactive intestinal polypeptide (VIP)

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Starvation causes changes in intestinal structure and function. Prolonged fasting in animals induces atrophic changes in the intestinal mucosal architecture (1–3), diminishes the intestinal disaccharidase activity (4, 5), and decreases the number of antral gastrin cells (6, 7). In starving man the ultrastructure of gastrin-producing G cells (8) and acid-producing parietal cells (9) shows signs of hypoactivity. Further, the pancreatic weight and exocrine function appear to be reduced by intraluminal gut starvation (10, 11). Gastrointestinal and pancreatic hormones are important in the regulation of these trophic and functional changes. It was therefore considered of interest to measure the plasma concentrations of vasoactive intestinal polypeptide (VIP), secretin, and gastrin, in addition to the serum concentrations of pepsinogen I (PG I) and human pancreatic polypeptide (hPP), during a 5-day period of absolute fasting. To evaluate the functional integrity of the gut after prolonged starvation, the postprandial pattern of release of these hormones was recorded both before and after the fasting period.

SUBJECTS AND METHODS

Eleven healthy young men (between 22 and 40 years of age; mean body weight, 76.9 ± 3.2 kg) gave their informed consent to participate in an experiment with 5 days of absolute fasting while performing their usual work at a research laboratory. They had free access to water. Before the experiment the subjects consumed a normal diet of approximately 35 kcal/kg body weight, consisting of 35% fat, 12% protein, and 53% carbohydrate. The subjects had a mean weight loss of 4.9 kg during the fast. Blood samples were drawn every morning during the period. To study the influence of starvation on the postprandial release of the gut hormones, a liquid meal (869 kcal) consisting of one egg and 80 g of Bio-

sorbin (Phrimmer, Erlanger, FRG) dissolved in 400 ml of milk was given in the morning after 12 h of fasting on day 1 and, after 5 days of fasting, on day 6. Blood was drawn from an antecubital vein 15 (−15) min and immediately before (0) and 30, 60, 90, 120, and 150 min after the start of the meal.

Blood samples for VIP were collected in heparinized ice-chilled tubes containing aprotinin (Trasylol®; 500 KIE aprotinin per milliliter blood), whereas plasma for gastrin and secretin were collected in EDTA tubes containing aprotinin and left on ice until centrifugation. Blood for preparation of serum was left at room temperature until centrifugation. Aliquots of plasma and serum were stored at −20°C until radioimmunoassay determination of VIP (12), secretin (13, 14), hPP (15), PG I (16), and gastrin.

In our hands the radioimmunoassay of VIP has a sensitivity of 2.5 pmol/l, an intra-assay precision of 9.8%, and an inter-assay variation of 20.2%. Plasma gastrin was measured by using a kit (Cambridge Nuclear) with a sensitivity of 5 pg/ml and showing 100% reactivity for G17, 29% reactivity for G34, and 0.1% cross-reactivity for secretin.

Serum glucose was determined by the hexokinase method, using a kit (Boehringer, Mannheim, FRG).

The results are presented as mean ± SEM. The significance of the differences induced by the fast was evaluated by the Wilcoxon's matched pairs signed-rank test. The integrated peptide response was calculated by trapeze integration from zero to the 150-min sample, with the prestimulatory concentration subtracted. p values less than 0.05 were considered statistically significant.

RESULTS

Vasoactive intestinal polypeptide

The plasma concentration of VIP increased during the fast, with a small rise ($p < 0.05$) on day 2 and a fivefold increase on day 6 (Table I). Refeeding on day 6 suppressed the plasma concentration of VIP after 30 min, and the plasma concentrations found in the control experiment were reached after 60 min (Fig. 1).

Secretin

The plasma concentration of secretin, measured by the method of Burhol & Waldum (14), increased during the fasting period and showed the highest level on day 2 (Table I). Ingestion of a meal induced on day 1 an increase ($p < 0.02$) in the plasma concentrations 60 min after onset and on day 6 throughout the experiment ($p < 0.03$) (Fig. 1). The postprandial plasma concentrations on day 6 were significantly higher than on day 1, except for the 150-min registration.

The plasma concentration of secretin in one fasting subject was compared as measured by the method of Schaffalitzky de Muckadell & Fahrenkrug (13) and by the method of Burhol & Waldum (14). By the former method (13) the fasting plasma concentration increased 19-fold after 5 days of fasting, and the change was suppressed by a meal (Fig. 2). By the method of Burhol & Waldum, however, the fasting plasma

Table I The fasting plasma concentrations of vasoactive intestinal polypeptide (VIP), secretin, and gastrin together with the fasting serum concentrations of pepsinogen I (PG I), human pancreatic polypeptide (hPP), and glucose were measured on separate days during 5 days of fasting

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
VIP, pmol/l	4.0 ± 1.0	7.9 ± 1.5*	15.1 ± 1.9‡	18.7 ± 2.1‡	19.7 ± 2.6‡	21.9 ± 3.1‡
Secretin, pmol/l	3.8 ± 1.0	7.5 ± 1.9†	6.1 ± 1.4†	5.7 ± 1.3†	5.6 ± 1.4†	5.7 ± 1.3*
Gastrin, pg/ml	24.1 ± 3.3	30.5 ± 3.1†	29.1 ± 3.2†	28.8 ± 3.1†	28.5 ± 3.9*	23.6 ± 2.9
PG I, ng/ml	77.4 ± 9.2	80.6 ± 6.2	81.0 ± 11.4	86.8 ± 9.1*	89.6 ± 12.3*	86.0 ± 12.5
hPP, pmol/l	10.4 ± 1.9	29.5 ± 4.4‡	36.8 ± 6.9‡	29.3 ± 5.7‡	39.8 ± 6.6‡	31.5 ± 6.5‡
Glucose, μmol/l	4.5 ± 0.1	4.1 ± 0.1*	3.5 ± 0.1*	3.4 ± 0.1‡	3.3 ± 0.1‡	3.3 ± 0.1‡

* $p < 0.05$ compared with day 1
† $p < 0.02$ compared with day 1
‡ $p < 0.005$ compared with day 1

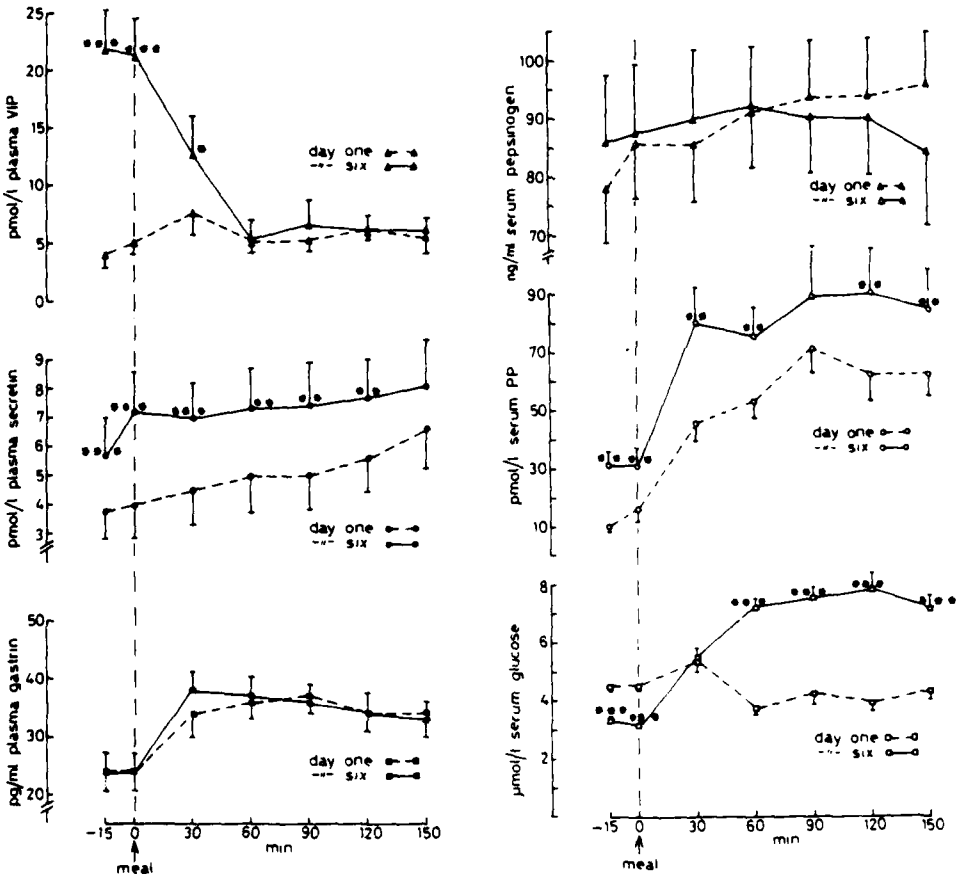

Fig. 1. The meal-induced changes in the plasma concentrations of VIP (pmol/l), secretin (pmol/l), and gastrin (pg/ml) together with the changes in the serum concentrations of PG I (ng/ml), hPP (pmol/l), and glucose (μmol/l) measured after 12 h of fasting (day 1) and after 5 days of fasting (day 6). $^{*}p < 0.05$ compared with day 1; $^{**}p < 0.02$ compared with day 1; $^{***}p < 0.005$ compared with day 1.

concentration of the same subject showed only a threefold increase, and there was no effect of refeeding (Fig. 2).

Gastrin

The plasma concentration of gastrin showed a small but significant increase from day 2 until day 5 during the fast (Table I). Postprandial plasma concentrations were unchanged after the prolonged fasting (Fig. 1).

Pepsinogen I

The serum PG I was slightly increased on day 4 and day 5 during the fast (Table I). After ingestion of the meal on day 1 the serum concentration rose significantly ($p < 0.02$) within 30 min and remained elevated throughout the experiment. On day 6 the meal induced an increase in serum concentrations for the 1st h after onset. The prolonged fasting did not influence the postprandial serum concentrations

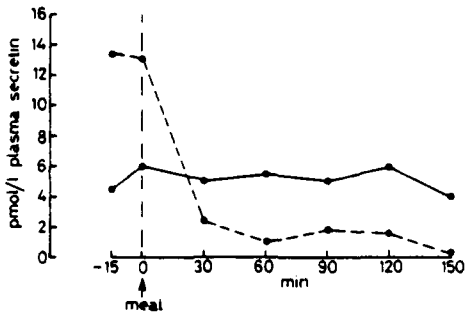

Fig. 2. The fasting and meal-induced changes in the plasma concentration of secretin in one subject exposed to 5 days of absolute fasting. The RIA method of Burhol & Waldum (——) and the RIA method of Schaffalitzky & Fahrenkrug (- - -) were used for the same subject.

Human pancreatic polypeptide

The serum concentration of hPP increased from three- to four-fold during the fast. Ingestion of the meal induced rapid and large increases in the serum concentrations in the control experiment and on day 6 (Fig. 1). The postprandial serum concentrations were higher on day 6 than on day 1 (Fig. 1). However, the mean integrated hPP response after the meal was not influenced by prolonged fasting (5.5 nmol·min/l and 7.2 nmol·min/l over 150 min on day 1 and day 6, respectively ($p > 0.05$)).

Glucose

The serum glucose concentration fell during the fast (Table I), and ingestion of the meal on day 6 induced glucose intolerance (Fig. 1).

DISCUSSION

This study shows that a prolonged fast in man affects both the basal level and the postprandial release of several gastrointestinal hormones.

VIP has a widespread neuronal distribution in the body but is mainly localized to the gut (17). It is considered to have a role as 'a polypeptide of substrate need' and has previously been found to increase during prolonged physical exercise (18, 19) and prolonged strain (20), leading to severe calorie deficiency. In accordance, our study showed that the plasma concentration of VIP was fivefold increased after 5 days of fasting and was suppressed by refeeding, which should indicate some metabolic influence of VIP during negative calorie balance in man. It is of interest that the plasma concentration of VIP has not been found to increase after fasting in obese subjects (21), who are suggested to have a state of impaired fat mobilization (22).

Plasma secretin determined by a double-antibody separation technique (14) was elevated during the fast, which is in accordance with prior studies by Henry et al. (23, 24) and Mason et al. (25) but in contrast to the results of Greenberg & Bloom (26). The discrepancy in the plasma concentration of secretin during prolonged fasting has been proposed to be explained by assay interference of lipolytic products (26). However, lipids have been shown to interfere only with the double-antibody technique (27). In our study one fasting subject showed an even more marked increase in plasma concentration when plasma secretin was determined by a radioimmunoassay method using charcoal separation (13). Immunosorption experiments show no interference of plasma lipolytic products in this secretin assay (Schaffalitzky de Muckadell, unpublished). It is therefore unlikely that the rise in plasma secretin may be attributed to unspecific interference with the separation procedure.

Using the method of Burhol & Waldum (14), refeeding caused a late rise in plasma secretin. On the other hand, in one fasting subject refeeding caused a sharp fall in plasma secretin when measured by the method of Schaffalitzky de Muckadell & Fahrenkrug (13). This observation is in agreement with our prior finding in healthy subjects during fasting accompanied by severe strain (28). The effect of refeeding on plasma secretin after a prolonged fast therefore appears to differ when using these two different radioimmunoassays. It is tempting to speculate that the plasma extraction procedure may be the cause of the discrepancy. Nevertheless, our results support the growing evidence that plasma secretin is increased after a prolonged fast.

In contrast to the finding by Uvnas-Wallensten & Palmblad (29) our study showed a small but

significant ($p < 0.01$) increase in the plasma concentration of gastrin during prolonged fasting. However, the response of plasma gastrin to a standard meal was not altered by starvation, in accordance with the findings of Henry et al. (24). Previously, adrenalin (30), growth hormone (31), and hypoglycemia (32) have been shown to enhance the gastrin release. It was therefore not surprising to register an increase in the fasting plasma concentration of gastrin in our study with hypoglycemia. Moreover, the levels of adrenaline (29) and growth hormone (33) are both expected to be increased by fasting. The discrepancy between our results and those of Uvnäs-Wallensten & Palmblad might be attributed to a difference in the gastrin molecular specificity measured by the two RIA methods.

Serum PG I reflects the total amount of pepsinogen I stored in the chief and in the neck cells of the gastric mucosa (34). There are no previous reports on PG I during a prolonged fast. The PG I values in this work are lower than previously reported by Waldum et al. (36) because we used a new standard with higher potency. The finding of a small increase in basal serum PG I on days 4 and 5 but not on day 6 in our study indicates that the chief cells and the neck cells are well preserved and not damaged as in gastritis (35). Waldum et al. (36) have shown a positive correlation between the basal serum concentration of gastrin and the serum concentration of PG I. In addition, secretin in physiological doses also increases serum PG I (37). The slight rise in serum PG I may therefore have been caused by a combined effect of the elevated gastrin and secretin concentrations. In accordance with previous findings (38, 39) a liquid meal induced a small increase in serum PG I. This postprandial rise was more short-lived on day 6, which could mean a washout of stored cell contents after a prolonged period of catabolism.

The three- to four-fold rise in serum level of hPP during prolonged fasting is well documented (40, 41). However, the elevated postprandial levels of hPP after starvation have not been registered before. Previously, the hPP response to a meal has been found unchanged after a prolonged period of total parenteral nutrition keeping the subjects euglycemic (42). This indicates that in our study the serum level of hPP is augmented as a consequence of the metabolic changes induced by the fasting rather than by the intraluminal starvation of the gut.

Finally, our study confirms previous findings (24, 43) that feeding after starvation causes hyperglycemia.

In conclusion, the study shows that the fasting and postprandial gut endocrine response in man is well maintained and rather increased after a prolonged starvation period.

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Responses of Vasoactive Intestinal Polypeptide, Secretin, and Human Pancreatic Polypeptide to Glucose during Fasting

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Øktedalen O, Opstad PK, Jorde R, Schaffalitzky de Muckadell OB. Responses of vasoactive intestinal polypeptide, secretin, and human pancreatic polypeptide to glucose during fasting. Scand J Gastroenterol 1984, 19, 59–64

The plasma concentrations of vasoactive intestinal polypeptide (VIP) and secretin and the serum concentration of human pancreatic polypeptide (hPP) were measured in nine healthy subjects during a 4-day fast. The fast induced a considerable increase in the concentrations of VIP and secretin but only a small increase in the concentration of hPP. The intravenous infusion of 50 g glucose and the oral ingestion of 50 g glucose temporarily suppressed the high concentrations of VIP and secretin. Conversely, hPP responded with a slight decrease in blood concentration after the intravenous infusion and with a modest increase after the oral ingestion. The study shows that glucose suppresses the high blood concentrations of VIP and secretin during starvation independent of the route of glucose administration. In addition, the results indicate that the blood concentration of hPP is not directly related to the blood glucose concentration during prolonged fasting.

Key words: Fasting; glucose; human pancreatic polypeptide (hPP); secretin; vasoactive intestinal polypeptide (VIP)

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The gastrointestinal peptides vasoactive intestinal polypeptide (VIP) (1), secretin (2, 3), and human pancreatic polypeptide (hPP) (4, 5) have possible physiological functions in the regulation of the exocrine pancreas. Moreover, the evidence that these peptides also have metabolic roles is accumulating. VIP and secretin, grouped together in the secretin family because of structural homology, exhibit lipolytic (6, 7), gluconeogenetic (8), and glycogenolytic (8) actions in vitro. Furthermore, both hormones are significantly elevated when man is in negative caloric balance (9, 10). The high plasma levels of VIP and secretin induced by fasting (9, 11) or by long-term physical exercise (12) are suppressed on refeeding. HPP serum levels are known to be stimulated by hypoglycemia (13, 14) and reduced by hyperglycemia (15, 16). These findings indicate a relationship between hPP and carbohydrate metabolism. Starvation in man alters glucose metabolism (17) and induces other metabolic changes (18). VIP-containing nerve fibers are mainly localized to the gastrointestinal tract (19). This, together with the finding of secretin producing S-cells in the duodenal and jejunal mucosa (20), makes it of interest to investigate whether the inhibitory effect of glucose on the blood concentrations of VIP and secretin during fasting is

dependent on a direct mucosal contact of glucose. Furthermore, we wanted to study the effect of starving on serum hPP.

MATERIALS AND METHODS

Nine healthy subjects (five men, median age 32 years, range 28–38 years; four women, median aged 32 years, range 21–43 years) gave their informed consent to participate in a 4-day absolute fast, while performing their daily work at a research laboratory. The subjects had free access to water and were given salt tablets during the fast. At the end of the period a median loss of 3.0 kg body weight (range 2.0–4.2 kg) was recorded.

Blood was obtained from an antecubital vein, and was drawn every morning during the period. On day 2 of the fasting period the subjects were given 230 ml of plain water, which was consumed within 2 min. The effect of 50 g glucose loading on the blood levels of VIP, secretin, and hPP was studied on 2 separate days during the fast. On day 3 an intravenous glucose infusion (*0.6 g/min* for 90 min, 12.5% solution) was given, and on day 4 a 50-g oral glucose tolerance test (50 g glucose in water, total volume of 230 ml, consumed within 2 min) was performed.

Blood was drawn 15, 60, and 90 min after water ingestion on day 2; after the glucose loading on days 3 and 4 blood was obtained at 15, 45, 60, 90, 120, 180, and 240 min. Blood samples for VIP and secretin were collected in heparinized ice-chilled tubes containing aprotinin (Trasylol®; 500 KIU aprotinin/ml blood). Blood for preparation of serum was left at room temperature until centrifugation. Aliquots of plasma and serum were stored at −20°C until determination of VIP (21), secretin (22), and hPP (23) by radioimmunoassay. Serum glucose concentration was determined by a hexokinase method (Boehringer, Mannheim, GFR).

Statistics. The results are presented as medians with the interquartile range in parentheses. The significance of differences induced by the fast on the glucose stimulations was elevated by the Wilcoxon's matched pair signed-rank test. P values less than 0.05 were considered statistically significant.

RESULTS

Vasoactive intestinal polypeptide. The plasma levels of VIP increased nearly threefold during the prolonged fast. The VIP concentration reached its highest point on day 2 and remained constant for the duration of the fast (Table I).

The ingestion of water on day 2 evoked no change in the plasma concentration of VIP (Fig. 1).

The intravenous infusion of 50 g glucose on day 3 and the oral ingestion of 50 g glucose on day 4 of the fasting period provoked the same profile of changes in the plasma level of VIP. In both cases VIP levels in plasma were decreased 45 min after glucose.

The greatest decrease occurred 90–120 min after loading.

Table I. The fasting blood concentrations of vasoactive intestinal polypeptide (VIP, pmol/l), secretin (pmol/l), human pancreatic polypeptide (hPP, pmol/l), and blood glucose (mmol/l) in healthy men (no. = 9) after an overnight fast (day 1), after a 46-h fast (day 2), after a 60-h fast (day 3), and after an 84-h fast (day 4)

	Day 1	Day 2	Day 3	Day 4
VIP	3.9	9.7**	9.6**	9.4**
	(3.0–5.1)	(7.0–12.0)	(7.0–10.3)	(8.3–12.1)
Secretin	1.0	3.6**	4.3**	4.7**
	(0.4–1.2)	(3.0–4.4)	(3.6–5.4)	(4.6–6.4)
HPP	7	9*	15*	9*
	(2–13)	(3–30)	(4–23)	(5–24)
Glucose	5.2	3.8**	3.4**	3.9**
	(5.0–5.4)	(3.7–4.1)	(3.1–3.7)	(3.8–4.2)

* $P < 0.05$, ** $p < 0.002$ compared with day 1. Median values with interquartile range in parentheses.

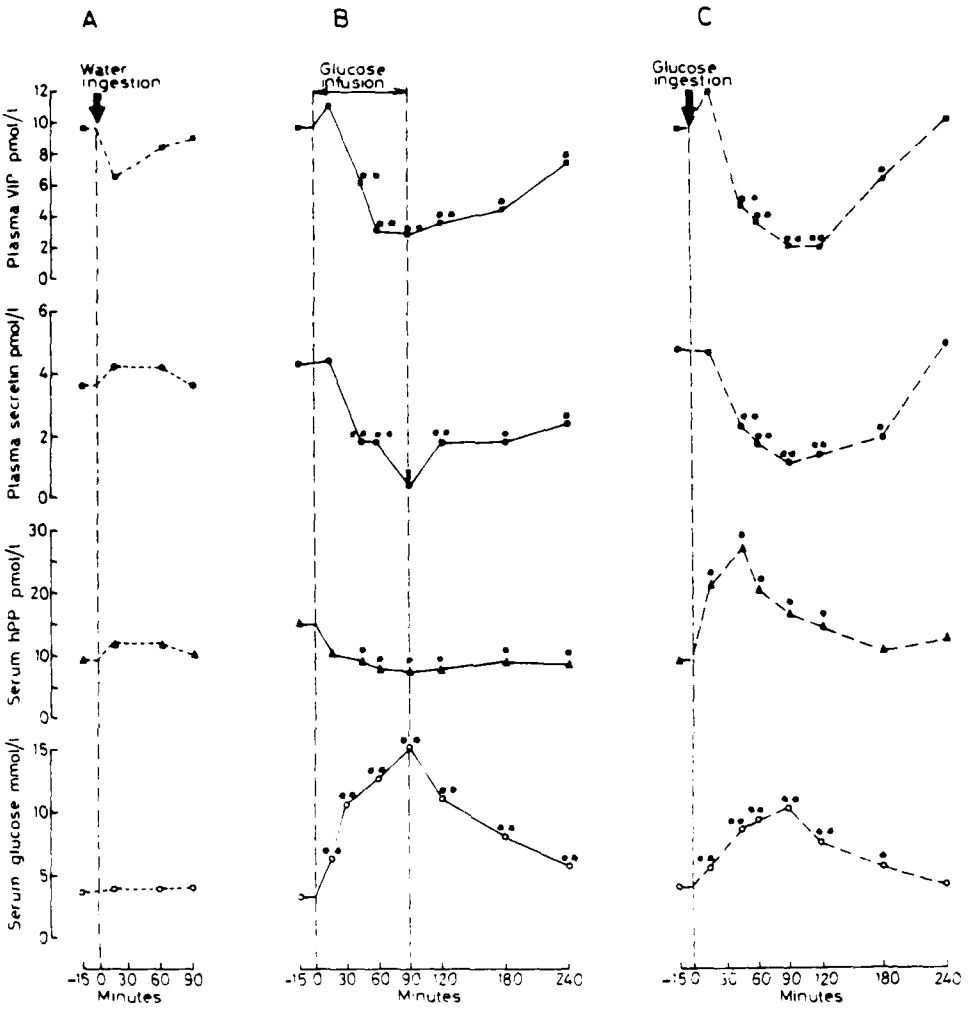

Fig. 1. The variations in blood concentrations of vasoactive intestinal polypeptide (VIP), secretin, human pancreatic polypeptide (hPP), and glucose after glucose stimulations in nine healthy subjects exposed to a 4-day fast. 1A Plain water (230 ml) was ingested after a 36-h fast. 1B Glucose infusion (0.6 g/min for 90 min, 12.5% solution) was administered after a 60-h fast. 1C A 50-g glucose tolerance test was performed after an 84-h fast. * $p < 0.05$ and ** $p < 0.002$ compared with pre-loading values. Median values.

The plasma level returned to the pre-loading value after 240 min in the oral tolerance test and nearly to the pre-loading level after 240 min in the infusion test.

Secretin. The fasting plasma level of secretin increased four- to five-fold during the fast, with no significant change in level among days 2, 3 and 4 (Table I).

Ingestion of water did not influence the level of secretin in plasma (Fig. 1). The intravenous glucose infusion on day 3 and the oral glucose ingestion on day 4 decreased the plasma level of

secretin within 45 min. A nadir was reached after 90–120 min, and thereafter the concentration increased (Fig. 1). The plasma level returned to the pre-loading value after 240 min in the oral tolerance test but not in the infusion test.

Human pancreatic polypeptide. The fast induced a slight increase in the fasting serum level of hPP, but no change in the levels among days 2, 3, and 4 of the fasting period (Table I) was found.

Intravenous infusion of glucose on day 3 of the fasting period was followed by a slight decrease in serum level of hPP (Fig. 1). A significant decline ($p < 0.02$) was obtained 45 min after onset, and a nadir was found after 90 min, when the glucose infusion was discontinued. On glucose withdrawal there was a small increase in the serum level of hPP, but the serum concentration did not return to the pre-infusion level during the test period. Ingestion of 230 ml of plain water on day 2 of the fasting period did not influence the hPP concentration in blood. However, oral glucose ingestion (50 g glucose in water, volume of 230 ml) provoked a prompt but moderate rise in hPP level, which peaked 45 min after ingestion and returned to pre-loading after 180 min.

Blood glucose. There was a decline in blood glucose level during the fasting period, with the lowest level on day 3 (Table I). The water ingestion on day 2 did not change the blood glucose. Intravenous infusion of glucose on day 3 caused a substantial rise in blood glucose and showed a 90-min peak of 15.6 mmol/l. Blood glucose declined after interruption of the glucose infusion, but the fasting level was not reached in the test period. After the oral glucose tolerance test performed on day 4 the blood glucose showed the same profile but with some lower levels than after the intravenous glucose infusion done on day 3.

DISCUSSION

The results of the study confirm prior reports that the plasma concentrations of VIP (9, 10) and secretin (9, 11) are significantly increased in starving man.

Prior data conflict with regard to what influence oral glucose (24–26) and intravenous glucose (24, 25) exert on the plasma concentration of secretin in normal man. However, it is a new finding that an intravenous infusion of 50 g glucose after 60 h of fast is followed by a substantial decrease in the high plasma concentrations of VIP and secretin. Oral ingestion of 50 g glucose after 84 h of fast caused a temporary decline in the plasma concentrations of VIP and secretin; this extends the findings of an earlier report on the suppressive effect of glucose on the plasma concentrations of VIP and secretin after starvation (9). These observations indicate that the inhibitory effect of glucose on the concentrations of VIP and secretin is not caused by the plain contact of the glucose solution with the intestinal mucosa and suggest that the effect is a consequence of the metabolic changes that occur after glucose loading in starving man. Similar to VIP and secretin in our experiment, FFA declines rapidly on refeeding after starvation (11). Moreover, Andrews et al. (9) have shown that VIP and secretin do not change during starvation in obese subjects, who are suggested to have a state of impaired fat mobilization (27). In the present study we noted a relationship between the stimulated blood glucose levels and the plasma levels of VIP and secretin. The lowest concentration of VIP and secretin coincided with the peak level of glucose, and both the blood glucose and the peptide concentrations returned nearly to pre-loading values at the same time. These reports and observations lend support to the speculation that VIP and secretin have a metabolic influence in humans who are in negative caloric balance. The results of our study could not be caused by nonspecific plasma artifacts, since control experiments have shown that glucose exerts no influence on the radioimmunoassay measurements of VIP (21) and secretin (22). Nor should the glucose effects be attributed to hyperosmolarity of the ingested glucose solutions since previous control experiments in man have shown no significant decline in plasma concentrations of VIP (28) and secretin (26) when stimulated by oral glucose and when the same radioimmunoassay methods for VIP (21) and secretin (22) were used. Furthermore, results from immunosorption studies indicate that there is no interference by plasma lipo-

lytic products in the VIP assay (Fahrenkrug, personal communication) or the secretin assay (Schaffalitzky de Muckadell, unpublished).

Our finding of high plasma concentrations of secretin during fasting could not be explained by an increase in the intraduodenal acidity. Most previous reports indicate that glycopenia does not initiate acid secretion under physiological conditions (29, 30), although Moore (31) has found a slight acid response to reduction in blood glucose beginning at normoglycemic levels. According to Moore (31), the increase in acid response is hardly measurable when the fasting blood glucose decreases by about 25% from the 5 pmol/l level. Thus, the acid stimulus is too small (32) to explain our hypersecretinemia.

The finding of a one- to two-fold increase in the fasting blood concentration of hPP is smaller than previously reported by our group, using the same analysis method (10), and by others (15, 33). No ready explanations can be offered for the difference. The effect of blood glucose variations on the PP secretion is well documented in nonstarving healthy man (13–15) but has not previously been studied during prolonged fasting. Although the glucose metabolism is highly disturbed in starving man (17), our results indicate that the PP response to exogenous hyperglycemia resembles that previously found during normal condition (13–15). The intravenous administration of glucose provoked a slight depression of hPP in blood, whereas an oral intake of glucose was followed by a prompt, modest and transient rise in the serum level of hPP. The latter observation should not be attributed to gastric distention, since the ingestion of an equal volume of plain water did not elicit hPP in blood. The response of hPP is dependent on the route of glucose administration, and the gastrointestinal tract appears important in provoking release after glucose intake. Since both of the glucose stimulations were associated with hyperglycemia, it is suggested that the glucose-induced changes in hPP levels during fasting are not mediated by a direct effect of glucose on the PP cell.

In conclusion, the study shows that starvation induces a large increase in the fasting plasma concentrations of VIP and secretin but only a slight increment in the fasting level of hPP. The elevated levels of VIP and secretin are depressed after glucose infusion and after oral glucose ingestion, which indicates some metabolic role for these two peptides during fasting. *HPP* responds with a small decrease to glucose infusion and with a slight, short-lived increase in the serum concentration after oral glucose ingestion. Thus, the release of hPP is not directly related to the blood glucose level during starvation.

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THE EFFECT OF PHYSICAL STRESS ON GASTRIC SECRETION AND PANCREATIC POLYPEPTIDE LEVELS IN MAN

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Short title: GASTRIC SECRETION AND HORMONES DURING STRESS

ABSTRACT

Twelve healthy subjects were exposed to a 4-day period of hard physical exercise, calorie supply deficiency, and severe sleep deprivation. The basal acid output (BAO), the sham-feeding induced acid output (MAO_{Sh}), and the pentagastrin stimulated acid output (MAO_{Pg}) were measured immediately after this stress period and in a control experiment performed several weeks later. The stress induced a 3-fold increase in the median BAO, an increase ($p<o.05$) in the MAO_{Sh}, which, however, was not significantly elevated when basal-subtracted. MAO_{Pg} was unchanged. In contrast to acid, pepsin output was not influenced by stress.

The human pancreatic polypeptide (hPP) level in serum increased 2-fold after the stress. The integrated hPP response induced by modified sham-feeding was higher ($p=0.02$) after the stress than in the control experiment.

The results show that physical stress has separated influence on the gastric secretion of acid and pepsin.

Key words : acid, gastric; gastrin; human pancreatic polypeptide (hPP); pepsin; sham-feeding; stress, physical.

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INTRODUCTION

Sham-feeding is a physiological procedure to test the cephalic phase of the gastric function. It has been shown (1) that the modified method of "chew and spit" represents an easy, safe and adequate way to induce vagal activation of the gastric secretion. Most investigators conclude that the acid secretion evoked by sham feeding is achieved by the direct vagal effect upon the parietal cells, and that the contribution of gastrin is of minor importance (2,3,4). Although the duodenal ulcer patients as a group secrete more gastric acid basally than healthy subjects (5,6,7), and the secretogenic capacity of the vagus is increased (8), there are conflicting data as to whether the acid response to modified sham-feeding differs in duodenal ulcer patients and normals (9,10).

Peptic ulcer disease has been considered to be influenced by stress. We have previously reported on basal hypersecretion of acid in man after having completed a 5-day period of physical stress (11). In that study, the blood level of gastrin was unchanged (11), while in another similar study the fasting and the stimulated serum levels of hPP were highly elevated (12). Several lines of evidence indicate that the serum level of hPP is dependent on a cholinergic vagal mechanism (13,14,15), and enhanced by cephalic vagal stimulation (3,16,17,18).

In general, the gastric secretion of acid and pepsin follow each other closely, and any dissociation of the acid and pepsin response indicate

an unequal stimulation or an altered sensitivity to stimulation of the secreting cells.

The present study was done to investigate the influence of prolonged physical stress on

1) the secretory potency of vagal and hormonal stimulation and selective effects on acid and pepsin secretion,

2) the release of gastrointestinal hormones after vagal stimulation.

MATERIALS AND METHODS

The subjects and the training course

Twelve military cadets (between 20 and 30 years of age) participated in a 4-day training course as a part of their training program. They were in excellent mental and physical condition with no history of gastrointestinal disease. The design of the training course was nearly the same as that previously described (11). The subjects were exposed to continuous, heavy exercise especially for the first three days of the training course. Due to continuous simulated combat activities, the subjects had only 2-4 hours of total sleep during the course.

The daily food intake varied on the different days of the course. The

subjects consumed k-ration (10 000 kJ) on the first day, a warm meal (3500 kJ) in the evening of the second day, slices of bread (2500 kJ) and half a cooked chicken (4400 kJ) on the third day, and a warm meal (3500 kJ) on the fourth day. As a consequence of the heavy physical exercise, the subjects were calorie deprived and showed a medium weight loss of 2.6 (1.5-3.5) kg during the course.

Conclusively, the multifactorial physical stress consisted of prolonged and heavy physical exercise, calorie supply deficiency and severe sleep deprivation.

Test procedures

In the morning of the fifth day of the course, after fasting for 12 hours and being abstinent from water for at least 3 hours, the subjects were taken out of the training course and immediately transported to laboratories of gastroenterology for gastric tests.

The control experiments were performed in the same laboratories several weeks later when the subjects had only foregoing school activities. A Levine tube was positioned under fluoroscopic control, and intermittent suction was applied. The stomach was thoroughly emptied, and the aspirate discarded. The gastric secretion of acid and pepsin were measured during three consecutive hours; one hour unstimulated, one hour during modified sham-feeding, and one hour after pentagastrin stimulation. In the period of basal secretion and in the period of modified sham-feeding, the gastric test was performed by instillation

(19). This procedure allows a much more reliable estimation of the pepsin release, the so called "non-active" pepsin secretion (20). Every 10 min period 200 ml of a weak acid solution (0.005 M HCl) containing polyethylenglycol (PEG, MW 4000) 2 g/ℓ and 0.9% NaCl was instillated into the stomach during 1½ min, was kept there for 5 min, before being aspirated during the last 3½ min of the period. The volume of gastric aspirate from each 10-min period was read to the nearest ml. The acid concentration of the aspirate was estimated by titration to pH 7.4 using an automatic titrator and pH meter (Autoburette, Copenhagen, Denmark).

The concentration of PEG was read spectrofotometrically, and the per cent recovery of PEG calculated. The pepsin concentration was measured by the human hæmoglobin method described by Berstad (21). Acid and pepsin outputs were calculated from the aspirate quantities corrected for incomplete recovery according to the PEG values and for the amount of acid instillated.

After a 60 min basal period, the subjects were served appetizing slices of bread. The food was continuously tasted, chewed and spat out during 10 min. Only small amounts of swallowed food particles were found in the gastric aspirate.

For comparison, the sham-feeding period was followed by a period of maximal acid stimulation induced by intramuscular injection of pentagastrin (6µg/kg body weight). During this period, the gastric

aspirate was continuously collected in six 10 min fractions without instillation of solution.

Blood samples were drawn every 10 min in the basal period and every 5 min for the first 30 min, whereas every 10 min for the last 30 min in the period of modified sham-feeding. Unfortunately, we were unable to obtain blood from one of the subjects. Blood for gastrin was collected in glass tubes containing EDTA, whereas blood for serum was allowed to clot before centrifugation. Aliquots of plasma and serum were stored at -20°C. Radioimmunoassays were employed for measurements of hPP (22) and gastrin (Cambridge Nuclear). The gastrin assay had a sensitivity of 5 pg/ml, and a cross reactivity of 100 per cent with G-17, 29 per cent with G-34, and 0.1 per cent with secretin. Serum glucose was determined by a hexokinase method (Boehringer, Mannheim, FRG).

Calculations and statistics

The basal acid output (BAO) and the basal pepsin output were defined as the sum of the acid and the pepsin outputs in the three last 10 min periods of the basal hour, multiplied by 2 to express results in mmol/h and mg/h, respectively. MAO_{sh} and MAO_{pg} represented the sum of the acid outputs in six consecutive 10 min periods of the sham-feeding hour and the pentagastrin stimulated hour, respectively. Similarly, the pepsin outputs were measured after modified sham-feeding and pentagastrin stimulation. Peak acid output (PAO) and peak pepsin output to pentagastrin stimulation were defined as the sum of the two highest

consecutive 10 min outputs of acid and pepsin, respectively, multiplied by 3 to express results in mmol/h.

The integrated response of human pancreatic polypeptide (hPP) after modified sham-feeding was calculated by trapez-integration from zero to the 15 min sample, with the basal concentration (median of the three last values in the basal period) subtracted.

The results were given as median with the interquartile range. Significance of differences was evaluated by the Wilcoxon's signed-rank test. $p<0.05$ was considered statistically significant.

RESULTS

The basal gastric acid secretion

The subjects showed a 3-fold increase in the basal acid output after the stress period, when compared to the control experiment (Fig 1). The increase was especially significant for the last 40 min of the test period when a plateau phase for the basal acid output was observed (Fig 2). The BAO/PAO ratio increased significantly ($p<0.01$) from 10.5(2.8-17.9) per cent in the control experiment to 22.9(17.2-36.4) per cent in the stress experiment (Fig 3).

The sham-feeding induced gastric acid secretion

The acid output induced by the modified sham-feeding (MAO_{sh}) measured over one hour (Fig 1) or in 10 min periods (Fig 2) were higher after the stress period than in the control experiment. However, the higher MAO_{sh} was mostly due to an increase in the basal acid output (BAO) since the basal-subtracted response (MAO_{sh}-BAO, not shown) and the $\frac{MAO_{sh}-BAO}{PAO}$ ratio (Fig 3) did not change after the stress period.

The maximal gastric acid secretion

The gastric acid secretion stimulated by pentagastrin (MAO_{Pg}) showed no change after a period of stress (Fig 1).

Pepsin output

The prolonged period of physical stress did not influence the "non-active" pepsin secretion in the basal period or the pepsin output after modified sham-feeding (Figs 1 and 2). Neither was the pepsin secretion after pentagastrin stimulation affected by stress (Fig. 1).

Hormones

The basal serum level of hPP was about 2-fold increased after the stress period (Table I). Modified sham-feeding induced a rapid, but modest rise in the serum level of hPP, and a peak was usually reached within the first 15 min (Fig 4). However, there was a marked variation in the response from one subject to another (Fig. 4). The

integrated hPP response for the first 15 min after modified sham-feeding was significantly higher ($p = 0.02$) after the stress period when compared to the control experiment.

There was a tendency towards lower basal plasma levels of gastrin after the stress period, but the difference was not statistically significant (Table I). Modified sham-feeding induced no significant change in the plasma level of gastrin either in the stress experiment or in the control experiment (Table I).

The fasting blood glucose level was not changed after the stress period, and the level was not altered after modified sham-feeding (Table I).

DISCUSSION

The results of this study confirmed our previous report (11) that the basal acid output (BAO) was 3 fold increased in man when exposed to a prolonged period of physical stress. The increase could not be due to a higher acid secretory capacity since the maximal acid output (MAO_{Pg}) or the peak acid output (PAO) were not changed after the stress period. The finding of an increase in the BAO/PAO ratio indicates either that a higher fraction of the parietal cell mass is active, or that more acid per parietal cell is secreted basally after a prolonged period of physical stress. The increase in BAO can not be caused by

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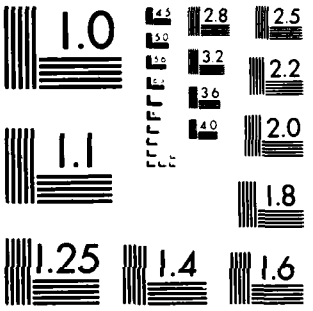

MICROCOPY RESOLUTION TEST CHART
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the gastrin hormone, since the circulating level of that hormone was not changed after the stress period. BAO is at least partially a function of the basal vagal activity (9,10), and the augmented BAO after stress could be explained by an increase in the vagal drive or by a specific hyper-sensitivity of the parietal cells, non-vagally mediated. The acid output induced by modified sham-feeding appears to be solely mediated through the vagal nerve (2,3,4), and sham-feeding has been considered a reliable test for evaluating the completeness of vagotomy (23,24). In our study, the acid output induced by the modified sham-feeding (MAO_{sh}) was increased, but the higher MAO_{sh} could be ascribed to the enhanced BAO after stress, since the basal-subtracted response MAO_{sh}-BAO was unchanged. In contrast to the augmented acid response, the pepsin output was not influenced by the stress either in the basal period, in the sham-feeding period, or in the pentagastrin stimulated period (Fig 1). Pepsin data have previously shown vast variations. Interestingly, there was a small distribution of pepsin outputs in the present study (Fig 2), so the pepsin secretion data appears most reliable. There is reason to suggest that the instillation technique is a better method for obtaining stable pepsin values.

The dissociation of the acid and pepsin response makes us suggest that the stress-induced gastric hypersecretion of acid is mediated through other pathways or other mechanisms than those affecting the pepsin release. The dissociation in the acid and pepsin response might be the result of heterogenous vagal activity, indicating that the vagal

influence of the parietal and the chief cells are conducted through different fibres or by different mediators.

In accordance with our previous finding (12,25), the fasting serum level of hPP was 2-fold increased after the stress. This increment was not caused by hypoglycemia since the fasting blood glucose was unchanged. hPP secretion has been proposed as an indicator of vagal tone (26). Our results of elevated hPP levels in the basal period after stress is therefore most likely caused by hyperactivity of the vagal nerve. This indicates that the vagus is triggered by the stress situation, and makes it more probable that also the parietal cells are influenced by the change in vagal activity. However, the effect appears different on the secretion of acid and pepsin.

The hPP response to modified sham-feeding was modest and varying, especially in the control experiment where the median hPP level after cephalic stimulation was not different from that in the basal period (Table I, Fig 4). It has been shown (3) that the hPP response to modified sham-feeding is closely related to the duration of the cephalic stimulation. Modified sham-feeding for 15 min appears necessary to get an increase in the serum hPP response (3). The short period of 10 min modified sham-feeding in our study may therefore explain the smaller hPP response than previously reported by others (3,16,17). Interestingly, the hPP response for the first 15 min period after modified sham-feeding was higher ($p = 0.02$) after stress than in the control experiment. This might indicate some hyperreactivity of the vagal nerve when excitated by the modified sham-feeding during stress.

The fasting gastrin level in plasma was unchanged after the stress period, in accordance with previous similar studies (11,12). In contrast to some authors (27,28,29), while in agreement with others (2,3,4), we observed no change of the gastrin level in plasma during modified sham-feeding either in the control experiment, or in the stress experiment. The results indicate a doubtful contribution of gastrin to the secretion of acid induced by modified sham-feeding after stress. Our results do not necessarily disagree with those of Feldman et al. (30) who found that the release of gastrin after modified sham-feeding was significant when the intragastric pH was kept at 5.0. Acidification to pH 2.5 abolished the gastrin response, and in our study the intragastric acidity, was kept lower than at pH 2.6.

In conclusion, the results of the present study indicate that the hypersecretion of acid after a period of physical stress in man must be mediated through other mechanisms or pathways than those affecting the pepsin release which is not influenced by stress. Though the stimulation of hPP after modified sham-feeding is modest and varying, the integrated response is higher than normally after physical stress.

Acknowledgements

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LEGENDS TO FIGURES AND TABLE

Figure 1 The outputs of gastric acid and pepsin basally, after modified sham-feeding, and after pentagastrin stimulation. The experiments were performed after having finished a 4-day period of physical stress (hatched column), and in a control experiment (open column). Median values and interquartile range.

* $p<0.05$ compared to the control experiment

* * $p<0.01$ compared to the control experiment

Figure 2 The outputs of acid and pepsin basally and after modified sham-feeding given as 10 min periods. The experiments were performed after having finished a 4-day period of physical stress (———), and in a control experiment (- - - -). Median values. The vertical bars show the interquartile range.

* $p<0.05$ compared to the control experiment

** $p<0.005$ compared to the control experiment

Figure 3 Individual results of the basal acid output (BAO), the modified sham-feeding induced acid output (MAO_{sh}), and the MAO_{sh}-BAO expressed as a ratio of peak acid output (PAO) measured in man (n=12) after having finished a 4-day period of physical stress (closed circles), and in a control experiment (open circles). Median values as horizontal lines.

Figure 4 Individual serum levels of human pancreatic polypeptide (hPP) measured in man (n=11) before and after a 10 min period of modified sham-feeding. The experiments were performed after a 4-day stress period (stress), and in a control study (control).

Right:

The integrated hPP response during the first 15 min after starting modified sham-feeding. Hatched column shows the stress experiment, open column the control experiment.

* $p = 0.02$ compared to the control experiment

Table I The plasma level of gastrin (pg/ml) and the serum levels of hPP(pmol/l) and glucose (mmol/l) before and after a 10 min period of modified sham-feeding which was started at time zero (0). The levels were measured in man (n=11) immediatley after having finished a 4-day period of physical stress (stress), and in a control experiment (control). Median values and interquartile range.

* $p<0.05$ compared to control

** $p<0.01$ compared to control

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		TIME (min)														
		– 50	– 40	– 30	– 20	–10	0	+ 5	+ 10	+ 15	+ 20	+ 25	+ 30	+ 40	+ 50	+ 60
GASTRIN	STRESS	30 (20–44)	30 (25–40)	30 (20–41)	20 (25–44)	20 (25–44)	31 (20–41)	33 (27–42)	31 (20–43)	32 (20–36)	32 (20–37)	32 (31–30)	20 (20–40)	32 (20–30)	32 (20–44)	33 (25–40)
	CONTROL	30 (30–43)	30 (33–44)	35 (20–41)	35 (33–43)	30 (27–42)	30 (20–40)	40 (34–43)	42 (33–40)	44 (30–45)	40 (30–40)	41 (32–43)	30 (30–43)	41 (20–44)	30 (30–41)	30 (30–44)
MPP	STRESS	11* (0–15)	11* (0–10)	14* (0–17)	12* (0–17)	11* (0–10)	14* (11–10)	23** (15–37)	22** (14–20)	10** (15–20)	17** (12–20)	15** (10–10)	14* (0–17)	10 (0–17)	15* (0–23)	14** (0–22)
	CONTROL	0 (4–0)	0 (4–0)	5 (4–7)	7 (4–0)	0 (5–0)	7 (0–0)	0 (5–15)	0 (7–0)	0 (7–10)	7 (7–0)	0 (7–0)	7 (7–7)	7 (0–13)	7 (0–13)	7 (7–0)
GLUCOSE	STRESS	4.0 (3.0–4.3)	4.0 (3.7–4.2)	4.0 (3.0–4.1)	3.0 (3.0–4.1)	4.0 (3.0–4.1)	3.0 (3.0–4.3)	4.0 (3.0–4.3)	3.0 (3.0–4.3)	4.1 (3.0–4.3)	4.1 (3.0–4.1)	4.1 (4.0–4.3)	3.0 (3.7–4.1)	4.1 (3.0–4.3)	4.0 (3.0–4.3)	4.2 (3.0–4.3)
	CONTROL	4.2 (4.1–4.3)	4.1 (4.0–4.2)	4.2 (4.0–4.3)	4.3 (4.1–4.5)	4.2 (3.0–4.4)	4.3 (3.0–4.3)	4.3 (4.1–4.4)	4.3 (4.2–4.3)	4.4 (4.1–4.5)	4.3 (4.1–4.5)	4.4 (4.1–4.4)	4.3 (4.2–4.5)	4.2 (4.0–4.4)	4.2 (3.0–4.4)	4.1 (4.0–4.0)

Table I

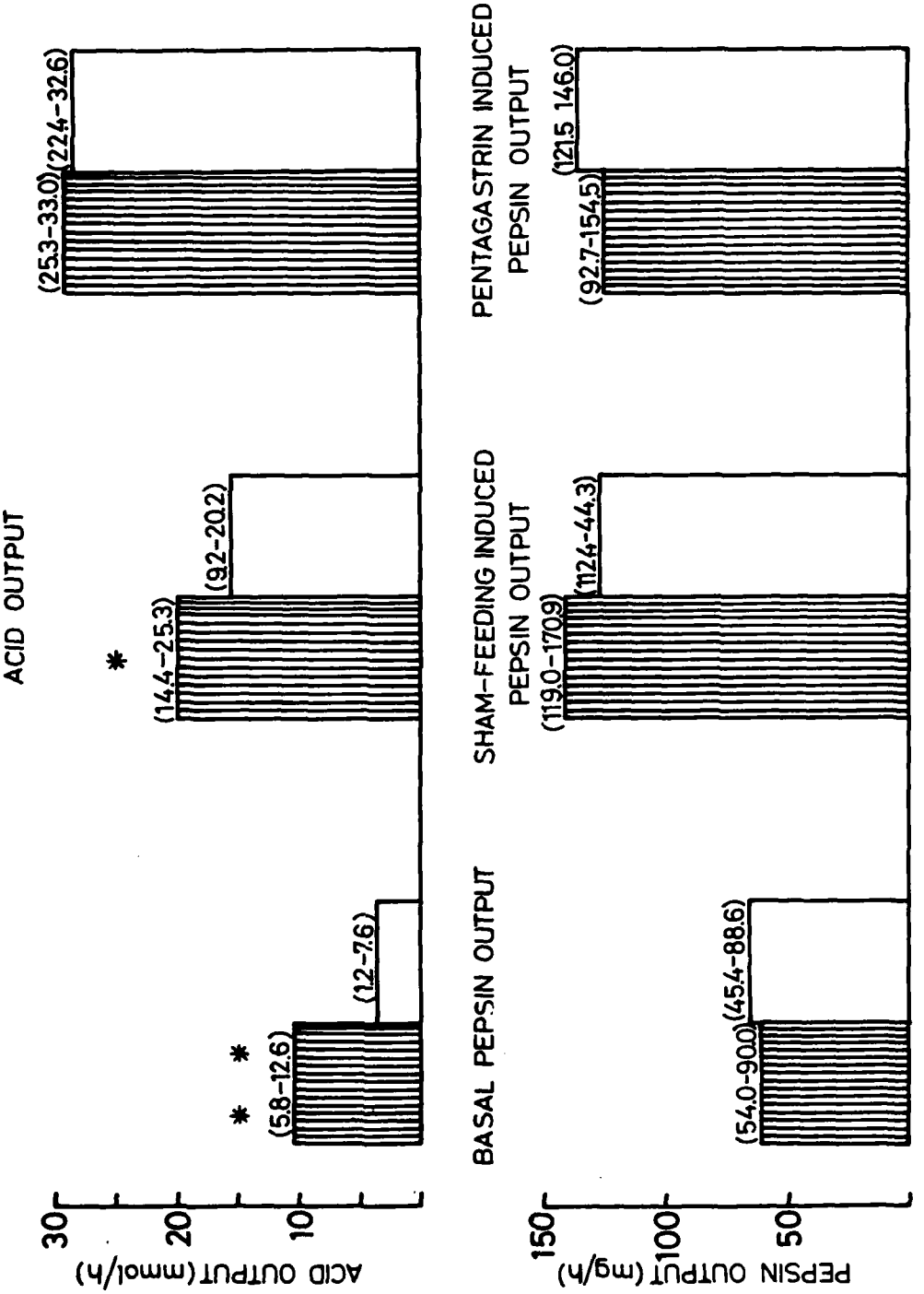
BASAL ACID OUTPUT
SHAM-FEEDING INDUCED ACID OUTPUT
MAXIMAL ACID OUTPUT
(5.8-12.6)
(12-7.6)
(14.4-25.3)
(9.2-20.2)
(25.3-33.0)
(22.4-32.6)
ACID OUTPUT (mmol/h)
30
20
10
BASAL PEPSIN OUTPUT
SHAM-FEEDING INDUCED PEPSIN OUTPUT
PENTAGASTRIN INDUCED PEPSIN OUTPUT
(54.0-90.0)
(45.4-88.6)
(119.0-170.9)
(112.4-44.3)
(92.7-154.5)
(121.5 146.0)
PEPSIN OUTPUT (mg/h)
150
100
50

Figure 1

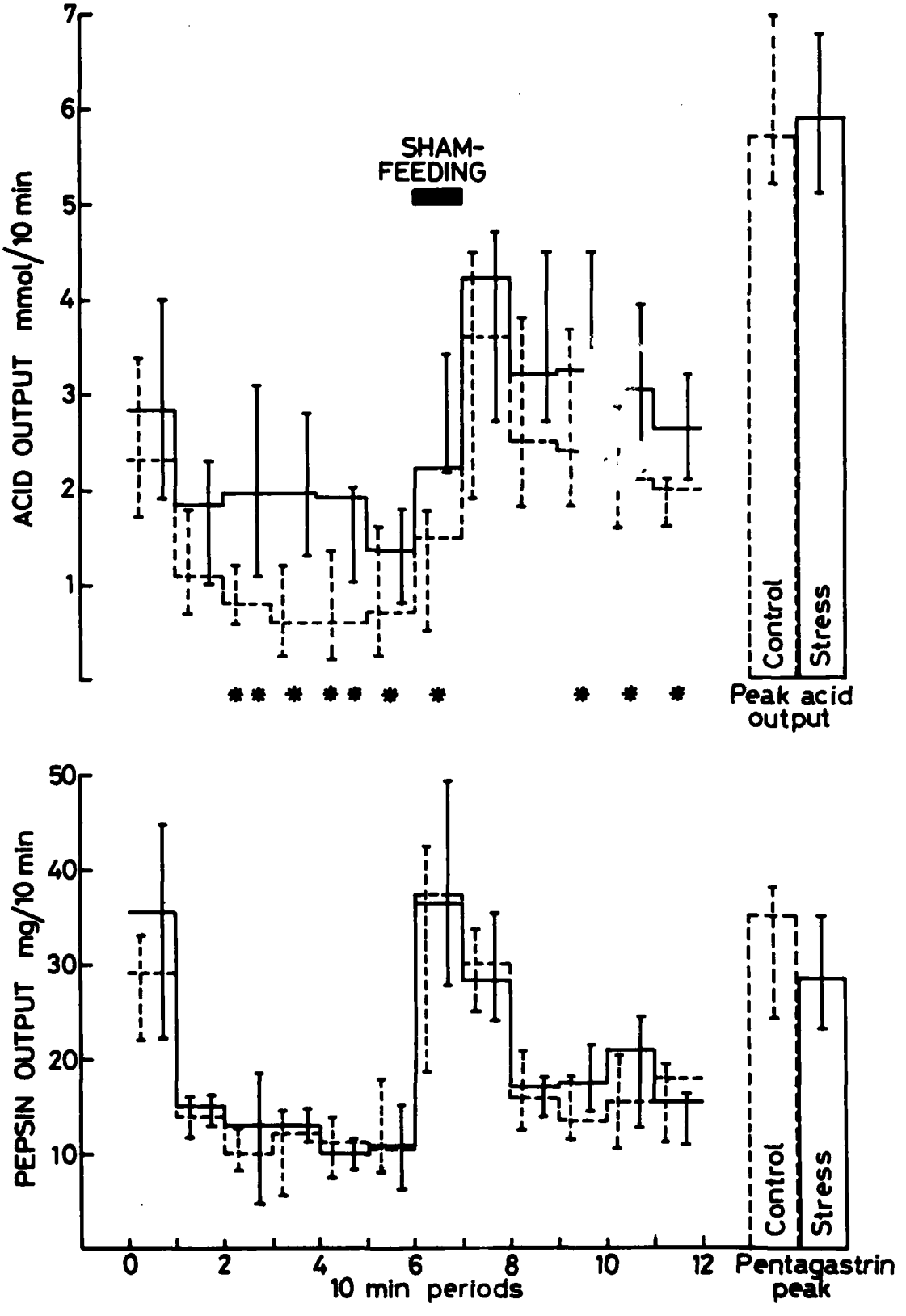
ACID OUTPUT mmol/10 min
SHAM-
FEEDING
Control
Stress
Peak acid
output
PEPSIN OUTPUT mg/10 min
Control
Stress
10 min periods
Pentagastrin
peak

Figure 2

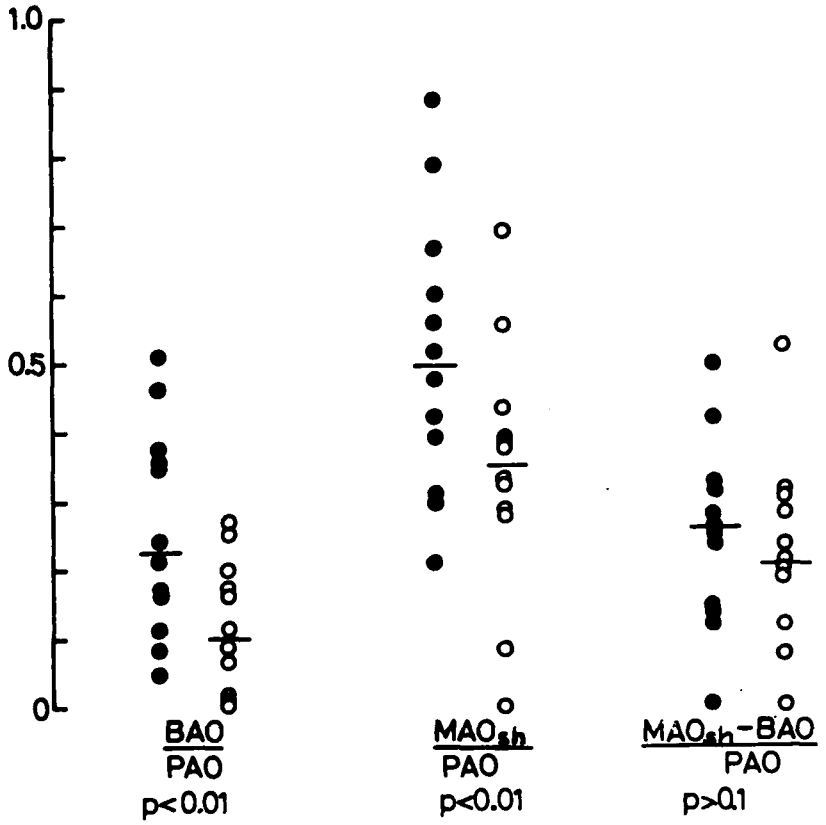

Figure 3

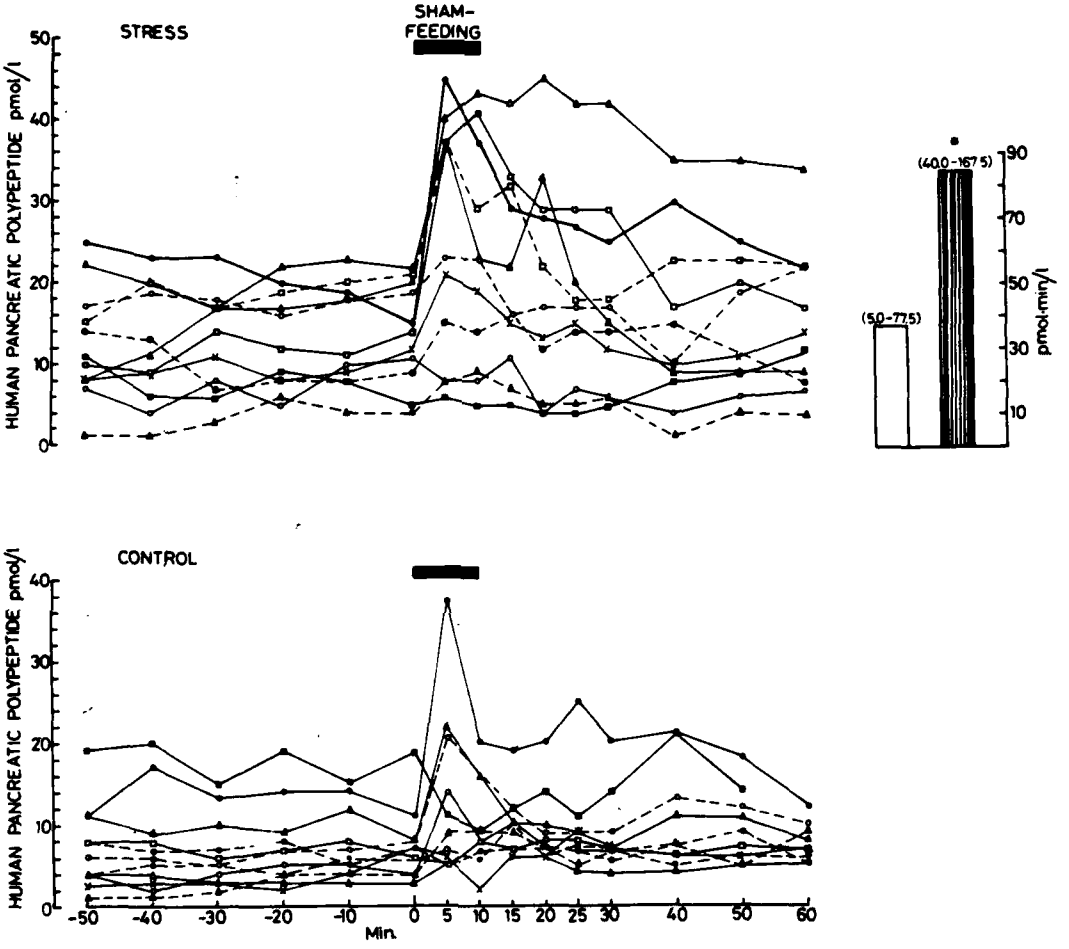
STRESS
SHAM-
FEEDING
HUMAN PANCREATIC POLYPEPTIDE pmol/l
50
40
30
20
10
0
(40.0-167.5)
(5.0-77.5)
90
70
50
30
10
pmol-min/l
CONTROL
HUMAN PANCREATIC POLYPEPTIDE pmol/l
40
30
20
10
0
-50
-40
-30
-20
-10
0
5
10
15
20
25
30
40
50
60
Min.

Figure 4